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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

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MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

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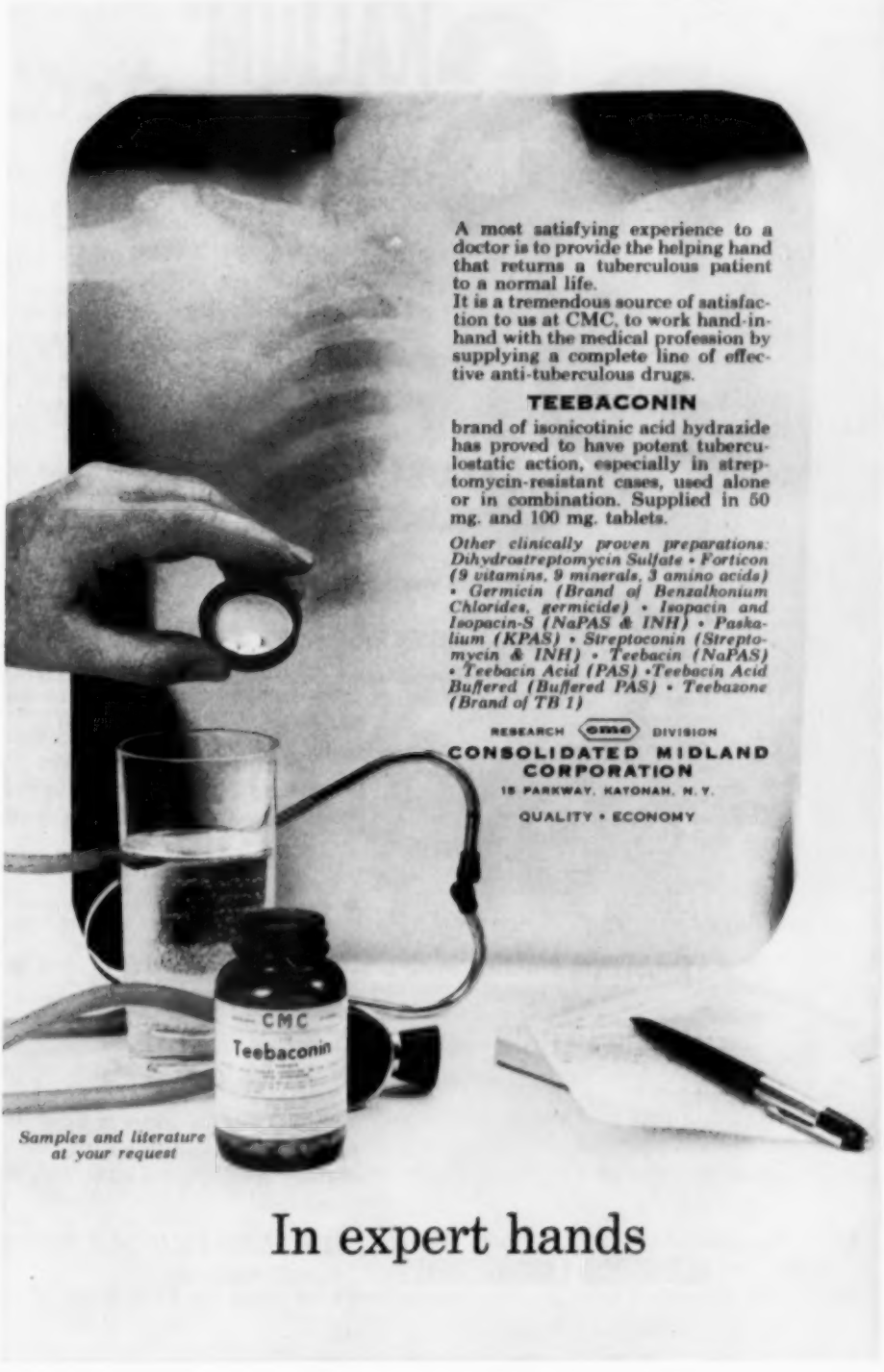
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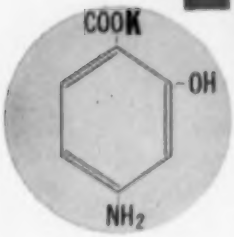

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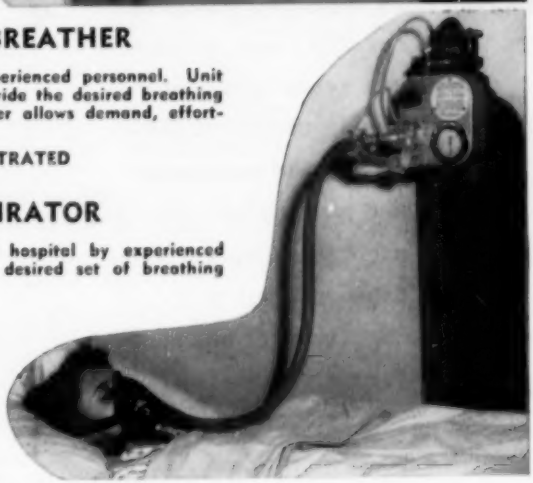

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New members of the Board as of July 1, 1956, are Dr. W. E. Adams, Dr. Max B. Lurie, Dr. Gardner Middlebrook, and Dr. George W. Wright, who have accepted three-year appointments.

ERRATUM

At the end of the seventh line of the Introduction to the article entitled "Experimental Air-Borne Infection" by Samuel Abramson, published in the March, 1956, issue of the REVIEW (volume 73, page 315), the words *living persons* should read *living animals*, as the tests were made in rabbits.

THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

VOLUME 74 *July, 1956* NUMBER 1

THE ACTION OF CORTISONE ON CORNEAL TUBERCULOSIS STUDIED WITH THE PHASE CONTRAST MICROSCOPE[1,2]

J. M. ROBSON AND K. A. DIDCOCK

(Received for publication February 28, 1956)

INTRODUCTION

The effect of cortisone (and corticotropin) on experimental tuberculosis and on the clinical course of the disease in man has been extensively investigated. Quite contradictory results have been published and it is clear that, depending on the experimental conditions, beneficial or deleterious effects may be produced.

As far as corneal tuberculosis in the mouse is concerned, it has been shown that the administration of cortisone in intact or in adrenalectomized animals produces a biphasic effect. The first phase consists of a slight beneficial action, lasting for approximately three weeks, during which the inflammatory and vascular reaction is somewhat reduced.

This is followed by the second phase, which consists of a deterioration of the cornea. A bleb forms in the center of the lesion which rapidly leads to perforation with caseous involvement of the cornea and the remainder of the eye. In addition, the disease spreads systemically, involving many organs, and many animals ultimately die from it (1).

The purpose of the present investigation was to study in greater detail the changes which occur in corneal tuberculosis under the influence of cortisone, using direct observation by means of the phase contrast technique (2).

METHODS

Mice were inoculated intracorneally with a suspension of bovine *Mycobacterium tuberculosis*, as used in previous investigations (3). Two separate experiments were performed. The inoculum consisted of a 1:100 dilution with Tween® saline of a culture ten days old in the first experiment and seven days old in the second. With a dilution of the culture of 1:1,000 the latent period before the appearance of a macroscopic lesion in untreated animals is of the order of ten days. In the present experiments, a dilution of 1:100 was used in order to facilitate the finding of organisms in the cornea; hence, as will be seen, the latent period was appreciably shorter.

[1] From the Pharmacology Department, Guy's Hospital Medical School, London, England.

[2] This study was aided in part by a grant (to J. M. R.) from the Medical Research Council, and in part by Guy's Hospital Endowment Fund.

In each experiment there were two groups of mice, i.e., one untreated and the other receiving 0.5 mg. of cortisone acetate per day subcutaneously; in both experiments the first injection was given shortly after corneal inoculation.

RESULTS

In the control animals, the corneal lesions developed as previously described, i.e., rapid cellular invasion with the formation of a core of cells and intracellular multiplication of bacilli with subsequent breakdown of macrophages, corresponding roughly to the appearance of a macroscopic lesion. In both the control groups, macroscopic lesions began to appear approximately the sixth day after inoculation.

In the cortisone-treated animals, the course of the infection in the cornea was very different. Twenty-four hours after inoculation, large clumps of bacilli were seen in the site of the needle track. There were few cells in contradistinction to the massive cellular invasion forming the core in the control animals. There were a few cells, however, and some of these appeared to contain a few organisms. In the remainder of the cornea there was a fair amount of cellular invasion including "gray" cells and some leukocytes. The central area also contained a few dots, again in contradistinction to the many seen in the equivalent site in the control cornea.

Forty-eight hours after inoculation, in the cortisone-treated animals the central distorted patch, representing the needle track, contained many clumps of bacilli but was practically free from cells, again in contrast to the control, in which there was massive cellular invasion of the site of inoculation. In the remainder of the cornea in the cortisone eye there was a good deal of cellular invasion, more marked than on the first day.

Four days after inoculation, quite a large area of the cornea was now occupied by masses of bacilli which were long, dark, and thin, and apparently actively multiplying. From this central mass, chains of bacilli were streaming towards the periphery of the cornea, sometimes in small cordlike aggregates. The central mass contained no intact cells but some cellular debris and some dots were present. On the following day, the bacillary multiplication in the cortisone eye appeared to have progressed still further so that the central mass now consisted essentially of aggregates of bacilli forming cords and palisades, each of these large aggregates representing very large numbers of organisms. There were also similar smaller aggregates of cord-forming bacilli. Again, massive migration of bacilli towards the periphery of the cornea was seen.

In figures 1 and 2 is illustrated the appearance of the central mass and of the bacilli migrating towards the limbus along a channel. In several places there were bacillary aggregates containing many cords and so arranged as to suggest strongly that they had overgrown a macrophage. Sometimes two or three aggregates with cellular debris were found together. The whole appearance suggested that there had been intense multiplication of the bacilli at the site of inoculation with practically no interference from cells. The bacilli had then migrated outwards, possibly along channels, and here and there met a few invading macrophages which had rapidly been overgrown and killed.

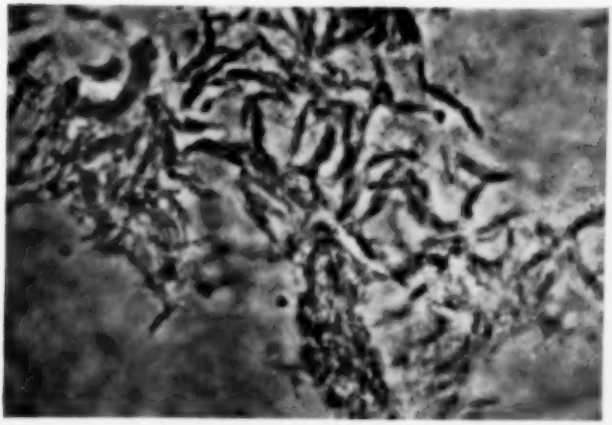

FIG. 1. Showing masses of bacilli, in cords and palisades, at the focus of infection in the cornea of a mouse inoculated five days previously and treated with cortisone. (Phase contrast × 1,000)

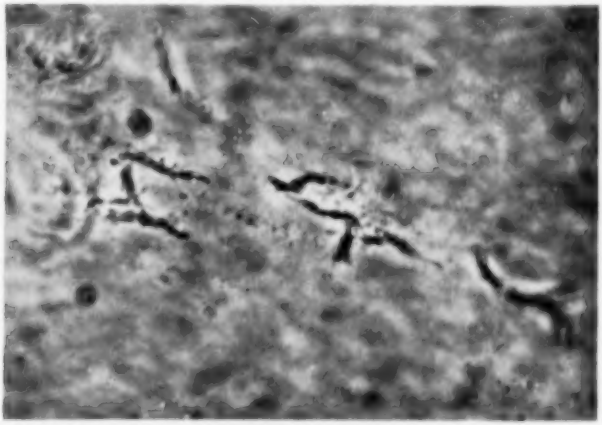

FIG. 2. Showing cords of bacilli in the peripheral part of the same cornea as in figure 1. (Phase contrast × 1,000)

Six days after inoculation, a number of control animals were beginning to show macroscopic lesions as did some of the animals treated with cortisone. One of these was used for microscopic observation. The macroscopic lesion consisted of a faint ring with a clear center. On microscopic observation the central mass was found to contain numerous bacilli in cords and palisades as on the previous day. There was also a good deal of cellular debris. Much of the remainder of the cornea was occupied by cords and even small palisades of bacilli quite free from any cellular structure or debris. At this stage, however, a new phenomenon was

seen, i.e., massive invasion of the cornea along one segment by cells, which were essentially "gray" cells. At the meeting place between the invading cells and the central mass of bacilli there was a lot of debris; also some reasonably intact cells containing bacilli were present. At this site there were also masses of bacilli which had obviously recently overgrown macrophages. The remainder of the peripheral area of the cornea contained relatively few cells.

These observations suggest that the cortisone treatment had, up to the previous day, markedly inhibited cellular invasion of the cornea allowing rapid free multiplication of the bacilli to form cords and palisades. By now, however, the stage had apparently been reached when some cellular invasion occurred in spite of the action of cortisone. The appearances suggested that, when these invading cells met the mass of multiplying bacilli, the latter were engulfed by the macrophages which were then killed and rapidly disintegrated at the meeting place between the central bacterial zone and the zone of cellular invasion.

Seven days after inoculation, the macroscopic lesions in the cortisone-treated eyes were beginning to show early bleb formation and, on microscopic examination, no further information could be obtained as no detail could be made out. It is to be noted that bleb formation in these experiments occurred earlier than in those described by Naguib and Robson (1), presumably because of the larger initial inoculum used.

DISCUSSION

The present experiments have shown that, when the cornea of the mouse is inoculated with *M. tuberculosis* and the animal is treated with cortisone, the initial massive cellular invasion with leukocytes and macrophages, which is the response to the inoculation in the untreated animals, no longer occurs. Very few cells are seen at the site of inoculation for several days. Very rapid bacillary multiplication occurs, however, and the bacilli observed are all situated extracellularly. Obviously the absence of macrophages and the failure of the bacilli to be phagocytosed has kept away one of the main mechanisms by which the body deals with the infection. It is also obvious that this rapid bacterial multiplication can occur in bacilli which have remained extracellular in the body.

As a consequence of this very rapid bacillary multiplication there is not only a large mass of bacilli at the site of inoculation, in cords and palisades, but these bacilli start migrating out towards the periphery of the cornea at an early stage of the infective process. This too is made possible by the absence of cellular interference. The migrating bacilli, which are found in various parts of the cornea and at various distances from the original inoculum, are again all extracellular. Moreover, the bacilli are not found in connection with debris, which might have suggested that they have been carried in cells which have been rapidly destroyed.

When this stage has been reached, there is obviously some attempt to counter it by massive cellular migration which occurs in spite of the continued action of cortisone. As was observed in the present experiments, however, this attempt to oppose the migration of the bacilli is unsuccessful and the migrating macrophages are rapidly destroyed. This has two consequences: First of all, the further invasion of the body by bacilli is made possible and the infection spreads to various

organs ultimately involving the death of the animal. This stands in contrast to what is observed in the absence of cortisone administration, when the process remains restricted to the cornea. The second consequence is that the local process in the cornea results in rapidly destructive changes involving the formation, first, of a "bleb" and, second, the destruction of the cornea and of the eye. It would thus seem that the main action of cortisone is on the cellular response to the inoculation.

Lurie and Zappasodi (4), who observed the effects of cortisone in rabbits inoculated with *M. tuberculosis* by inhalation, found that the lesions in the lungs were very numerous and swarming with bacilli. They attributed the effect of cortisone to two main factors as follows:

> A decrease in the permeability of capillaries which presumably limits the migration of phagocytic cells towards the site of infection. Such an effect may help to explain the failure of cellular migration from the limbus to the corneal site of infection, seen in the present experiments.
>
> An action of cortisone in depressing the capacity of the phagocytes to inhibit the growth of the bacilli in their cytoplasm. This was seen in the present experiments, too, at the sixth day after inoculation when the cells migrating into the cornea were rapidly disintegrated by the multiplying bacilli.

Cortisone may have had other effects which have not yet been studied in the experimental system used in the present investigation. For example, the presence of cortisone may alter the virulence of the organisms. Moreover, there is a good deal of evidence that cortisone interferes with immune processes and this too may have contributed to the rapid development of the infection in the cortisone-treated animals.

Finally, it would obviously have been of interest to follow the processes in the cornea by a method which allows continual observation. The present conclusions are based on the appearances seen in the cornea at fixed stages of the investigation and their validity would be further strengthened if they could be continuously observed. Attempts are at present being made to devise a technique by which development of the tuberculous infection with the fate of the organisms can be continuously followed.

SUMMARY

The effect of cortisone on the course of corneal tuberculosis in mice was followed by means of the phase contrast microscope. There is a striking inhibition of cellular invasion of the site of inoculation and a rapid multiplication and spread of the bacilli. Later on some cellular invasion does occur but fails to stop the spread of the infection which disseminates systemically and ultimately kills the animals. Locally the intense bacillary multiplication is accompanied by "bleb" formation and the destruction of the eye.

SUMARIO

La Acción de la Cortisona sobre la Tuberculosis Corneal Estudiada con el Microscopio de Fases de Contraste

El efecto de la cortisona sobre la evolución de la tuberculosis de la córnea fué observado por medio del microscopio de fases de contraste. Nótanse notable inhibición de la invasión

celular en el sitio de inoculación y rápida multiplicación y difusión de los bacilos. Más tarde, sobreviene alguna invasión celular, pero no contiene la propagación de la infección que se disemina sistemáticamente y por fin mata a los animales. Localmente, la intensa multiplicación bacilar va acompañada de formación de "ampollas" y de destrucción del ojo.

RESUME

Action de la cortisone sur la tuberculose cornéenne étudiée avec le microscope à contraste de phase

L'effet de la cortisone sur l'évolution de la tuberculose cornéenne de la souris était observé à l'aide du microscope à contraste de phase. Il se produit une inhibition saisissante de l'invasion cellulaire au point d'inoculation une prompte multiplication et une dissémination rapide des bacilles. Ultérieurement, un certain degré d'invasion cellulaire se produit mais ne suffit pas à arrêter le processus de dissémination de l'infection qui envahit l'organisme et finit pas tuer l'animal. Localement, cette multiplication bacillaire intense s'accompagne de formation de bulles et de destruction de l'oeil.

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THE EFFECTS OF ISONIAZID TREATMENT ON THE TUBERCULIN REACTION AND ON THE HEALING OF BCG VACCINE-INDUCED ULCERS[1]

JOSEPH D. ARONSON, HELEN C. TAYLOR, AND DANIEL L. KIRK

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INTRODUCTION

Studies dealing with the effect of antimicrobial agents on the tuberculin reaction of tuberculous patients and tuberculous guinea pigs have shown equivocal results.

Feldman, Hinshaw, and Mann (1) as well as Steenken and Wolinsky (2) found that tuberculous guinea pigs treated with streptomycin showed a decrease or loss of tuberculin hypersensitivity. On the other hand, under different experimental conditions, Steenken and Pratt (3) noted that tuberculous guinea pigs treated with streptomycin continued to retain their tuberculin hypersensitivity. Woodruff and associates (4) found that tuberculous patients initially anergic to tuberculin who responded favorably to streptomycin therapy showed an increase in their tuberculin hypersensitivity. The tuberculin reaction of tuberculous guinea pigs treated with iproniazid became atypical or negative according to Steenken and Wolinsky (5).

A report from the Veterans Administration Hospital, Minneapolis, Minnesota, (6) indicates that, of twenty-two tuberculous patients who reacted to 0.00002 mg. of PPD-S tuberculin and who were treated with isoniazid for three to four months, nine patients subsequently failed to react to tuberculin, three showed a decrease of tuberculin hypersensitivity, while in the other ten patients the level of hypersensitivity was unchanged. Criep and Levin (7), however, claim that the level of tuberculin hypersensitivity was not modified in patients treated with isoniazid for one month. Similarly, Hewell and Suyemoto (8) found that, although the tuberculin reaction of tuberculous patients treated with isoniazid for one to four months did not differ significantly from that observed in tuberculous patients who did not receive isoniazid, the level of tuberculin hypersensitivity increased when treatment with isoniazid was continued for four to six months.

Variations and loss of sensitivity to tuberculin have been observed in the course of some diseases. These changes are usually of a transitory nature, and tuberculin hypersensitivity returns with the improvement or recovery of the patient. The tuberculin reaction which may be slight or absent in those suffering from far advanced tuberculosis or tuberculous meningitis may return in those whose condition improves under antimicrobial treatment.

Dahlstrom (9) found that with few exceptions the tuberculin reaction per-

[1] From the Henry Phipps Institute, University of Pennsylvania, Philadelphia, Pennsylvania; Bureau of Tuberculosis Control, Department of Health, Commonwealth of Pennsylvania, Harrisburg, Pennsylvania; and Elwyn Training School, Elwyn, Pennsylvania.

sisted in those with a high level of tuberculin hypersensitivity but tended to revert to negative in those with a low level of hypersensitivity.

In view of the comparative instability of the tuberculin reaction in those with a low level of hypersensitivity, it seemed reasonable to assume that changes or reversion of the tuberculin reaction could be more readily induced in this group. Since the intracutaneous administration of BCG vaccine in tuberculin-negative persons gives rise to a low level of tuberculin hypersensitivity, it might be expected that the tuberculin reaction of such vaccinated persons would more likely be modified by isoniazid or other antimicrobial agents than would the reaction of those with a high level of tuberculin hypersensitivity.

MATERIALS AND METHODS

A group of 116 white male and female mentally retarded patients of the Elwyn Training School who had failed to react to the intracutaneous injection of 0.01 mg. of Old Tuberculin (OT) received simultaneously 0.1 mg. of freshly prepared BCG vaccine in the skin, over the region of the deltoid, and 0.1 mg. of OT in the skin of the volar surface of the forearm. A second group of 122 persons who had received BCG vaccine from one to five times previously, but who failed to give a definite reaction to 0.1 mg. of OT at the time of the present study, were again injected with BCG vaccine in the same manner as the first group. Of those who had not been vaccinated previously, 80 per cent ranged in age from eight to twenty years while, among those who had been vaccinated repeatedly, only 35 per cent were from eight to twenty years old.

Six weeks following vaccination the site of injection was examined. Each person showed a local ulcer of varied size associated in three instances with slight transient regional adenopathy. In order to establish at this time their level of post-vaccinal tuberculin hypersensitivity, all were retested with 0.01 and 0.1 mg. of a freshly diluted sample of the same OT used in the initial tests. Of the 116 persons who had received their initial injection of BCG vaccine six weeks previously, 79.2 per cent gave a definite post-vaccinal reaction to 0.01 mg. of OT, 97.4 per cent reacted to 0.1 mg. of OT, and 2.6 per cent gave a doubtful reaction to the larger dose of OT. On the other hand, of the 122 persons who had been vaccinated previously from one to five times, only 32.8 per cent gave a definite reaction to 0,01 mg. of OT, 78.7 per cent reacted to 0.1 mg. of OT, 18 per cent gave a doubtful reaction, and 3.3 per cent failed to react to the larger dose of OT.

Because of the differences in the level of the post-vaccinal tuberculin hypersensitivity observed in the two groups, the effect of isoniazid on the tuberculin reaction was studied in representatives of both groups. One half of each group selected at random received daily by mouth 5.0 mg. of isoniazid per kg. of body weight, while the remaining half received no treatment and served as a control group. Treatment with isoniazid was instituted 47 days after the injection of BCG vaccine and was continued for 64 days. The isoniazid-treated and the control subjects were again tested 111 days after the administration of the BCG vaccine with 0.01 and 0.1 mg. of fresh dilutions of the sample of OT used throughout these studies. The tuberculin reaction was interpreted without the examiner's knowing which were treated and which were control subjects.

OBSERVATIONS

The results of the tuberculin test six weeks after vaccination and before treatment with isoniazid was instituted are compared in table 1 with the results of the tuberculin test 111 days after vaccination following 64 days of treatment with isoniazid.

It will be noted in table 1, that, among those in the category of initial vaccina-

TABLE 1

THE EFFECT OF ISONIAZID TREATMENT ON THE TUBERCULIN REACTION OF BCG-VACCINATED PERSONS

Category	Number Tested	Tuberculin Reaction 42 Days After Vaccination and Prior to Treatment with Isoniazid											
		Reaction to 0.01 Mg. OT						Reaction to 0.1 Mg. OT					
		Positive		Degree of Reaction				Positive		Degree of Reaction			
				2+ and 3+ Reactions		Doubtful*				2+ and 3+ Reactions		Doubtful*	
		Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Initial vaccination control subjects	55	45	81.8	42	93.4	10	18.2	55	100.0	55	100.0	0	—
Initial vaccination treated subjects	61	47	77.0	39	83.0	12	19.7	58	95.0	58	100.0	3	5.0
Repeated vaccination control subjects	58	16	27.6	12	75.0	35	60.3	45	77.6	45	100.0	10	17.2
Repeated vaccination treated subjects	64	24	37.5	19	79.2	33	51.6	51	79.7	51	100.0	12	18.7

Category	Number Tested	Tuberculin Reaction 111 Days After Vaccination and After 64 Days of Treatment with Isoniazid											
		Reaction to 0.01 Mg. OT						Reaction to 0.1 Mg. OT					
		Positive		Degree of Reaction				Positive		Degree of Reaction			
				2+ and 3+ Reactions		Doubtful*				2+ and 3+ Reactions		Doubtful*	
		Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Initial vaccination control subjects	55	40	72.7	26	65.0	10	18.2	53	96.4	52	98.2	2	3.6
Initial vaccination treated subjects	61	40	65.6	24	60.0	13	21.3	57	93.4	53	93.0	3	5.0
Repeated vaccination control subjects	58	16	27.6	7	43.8	24	41.4	43	74.2	35	81.4	13	22.4
Repeated vaccination treated subjects	64	15	23.4	7	46.7	24	37.5	54	84.4	43	79.6	10	15.6

* Based on the number tested

tion, approximately the same percentage of those persons selected at random to receive isoniazid and those selected as control subjects reacted to 0.01 and 0.1 mg. of OT six weeks after vaccination. A comparison of the tuberculin reaction, in the control and isoniazid groups 111 days after vaccination and after 64 days of treatment with isoniazid shows that 9.1 per cent fewer of the control subjects and 11.4 per cent fewer of the treated persons reacted to 0.01 *m.g.* of OT than reacted 42 days after vaccination and before isoniazid treatment was instituted.

That the decrease in the level of tuberculin hypersensitivity is a function of time and not due to treatment with isoniazid is indicated by the fact that, not only did the percentage who reacted to 0.01 mg. of OT decrease almost to the same degree in the control group as in the treated group, but the percentage of 2- and 3-plus reactions decreased even more significantly in both groups. Thus, in the 69 days between the tuberculin tests, the 2- and 3-plus reactions decreased by 28.4 per cent in the control group and by 23 per cent in the isoniazid-treated group. On the other hand, the decrease in the percentage of positive reactions and in the percentage of 2- and 3-plus reactions to 0.1 mg. of OT in the 69 days between the tuberculin tests was slight for both controls and isoniazid-treated persons.

Among the control and treated groups in the category of repeated vaccinations, the group selected at random to receive isoniazid showed 10 per cent more reactions to 0.01 mg. of OT 42 days after vaccination than did the group selected as controls. However, at that time approximately the same percentage in both groups reacted to 0.1 mg. of OT. At the end of the 69-day period between the two tuberculin tests, approximately the same percentage of the controls reacted to 0.01 mg. and 0.1 mg. of OT as reacted 42 days after vaccination.

Of those treated with isoniazid for 64 days and tested 111 days after vaccination, 14.1 per cent fewer reacted to 0.01 mg. of OT than reacted before treatment was instituted 42 days after vaccination; while 4.7 per cent more reacted to 0.1 mg. of OT after treatment with isoniazid. This decrease in the percentage who reacted to 0.01 mg. of OT is not significant statistically and is not necessarily related to treatment with isoniazid. Tuberculin hypersensitivity among those who have received a single or repeated BCG vaccination tends to decrease with time. It will be noted from table 1 that, among those who had received repeated vaccinations, the reduction in the percentage who gave definite reactions occurred to the same degree among the controls as among the isoniazid-treated group. Among the control group, 111 days after vaccination, 31.2 per cent and 18.6 per cent fewer gave a well-marked 2- or 3-plus reaction to 0.01 mg. and 0.1 mg. of OT, respectively, than they did 42 days after vaccination. Among the treated group 111 days after vaccination, 32.5 and 20.4 per cent fewer gave a 2- or 3-plus reaction to 0.01 and 0.1 mg. of OT, respectively, than they did 42 days after vaccination.

Effect of Isoniazid on Ulcers Induced by BCG Vaccine

In order to establish the relative value of the tuberculin reaction and the local reaction to BCG vaccine in detecting hypersensitivity, Aronson and McGettigan (10) correlated the local reaction to tuberculin and to BCG vaccine 48 hours after injection. They found that, of those who were negative or doubtful to 0.01 mg. of OT but positive to 0.1 mg. of OT, 63.8 and 64.4 per cent, respectively, showed an acute inflammatory reaction at the site of vaccination (Koch phenomenon). In the present study, the writers have found that, of 116 persons who had not received BCG vaccine previously and who failed to react to 0.01 mg. of OT, 28 (24.1 per cent) showed an acute inflammatory reaction at the site of

BCG vaccination (Koch phenomenon); and that 25 of the 28 (89.3 per cent) reacted to 0.1 mg. of OT given simultaneously with the vaccine. On the other hand, of the 122 persons who had received previously one or more injections of BCG vaccine, 103 (84.4 per cent) showed a Koch phenomenon and, of this group of 103 persons, only one (0 9 per cent) showed a definite reaction to 0.1 mg. of OT; 84 (81.6 per cent) gave a doubtful reaction; and 18 (17.5 per cent) failed to react.

This study offered a unique opportunity to observe under control conditions the effect of isoniazid on the local ulcer induced by the intracutaneous injection of BCG vaccine in two groups of persons, those receiving their initial BCG vaccination and those who had received previously one or more injections of BCG vaccine.

Previous studies (11) have shown that even under comparable conditions of dose, preparation of vaccine, site of injection, and depth of injection, definite variations in the intensity and character of the local reaction follow the injection of BCG vaccine. These variations may be due, in part, to differences in individual resistance or to a lack of uniformity in the administration of the vaccine. The BCG vaccine used in this study was freshly prepared 48 hours previously, was protected from direct sunlight, and kept at 4°C. until used. All injections were made by the same person and, immediately after each injection, a small white wheal was noted. The local lesion induced by the BCG vaccine was observed after 48 hours, again after 36 days, and at fortnightly intervals beginning 57 days after vaccination and ending 113 days after vaccination.

In those persons who had not previously received BCG vaccine and who failed to react to 0.01 and 0.1 mg. of OT, the intracutaneous injection of BCG vaccine was followed 48 hours later by a sharply defined, slightly elevated, painless papule (3 to 7 mm. in diameter), surrounded by a narrow red areola but with no associated significant edema. After 36 days a sharply defined ulcer was noted, ranging in size from 4 to 8 mm. in diameter and from 1 to 2 mm. in depth, covered by a dry scab of varying thickness. The base of the ulcer was usually covered with normal appearing granulation tissue but occasionally it was covered by a dirty gray exudate. The skin and subcutaneous tissue surrounding the ulcer were normal in appearance and density. When observed 57 and 71 days after vaccination, many of the ulcers were approximately the same size as they were after thirty-six days but tended to be more superficial. In some instances the ulcer had healed leaving a small, pale red, depressed scar. When observed 85 days after vaccination, the ulcers had healed in the great majority of persons, while 113 days after vaccination only an occasional person continued to show a small ulcer which discharged a small amount of pale yellow serous exudate on slight pressure.

In those persons who had not previously received BCG vaccine but who showed a Koch phenomenon following their initial vaccination, the ulcers when observed 36 days after vaccination were larger and deeper than the ulcers of those who did not show a Koch phenomenon. When observed after 57 days, several had healed; and only an occasional ulcer was noted 71 days after vaccination. All ulcers had healed 85 days after vaccination, leaving a pink depressed

scar which was somewhat larger than that observed in those who did not show a Koch phenomenon.

The gross appearance and rate of healing of the ulcers of those treated with isoniazid were not significantly different from these phenomena in the untreated controls, and the isoniazid-treated subjects could not be differentiated from the controls by the appearance of the ulcer.

Among the 122 persons who had received BCG vaccine from one to five times previously, the reaction to BCG vaccine was more intense than the slight local inflammatory reaction which followed the initial BCG injection in those who failed to react to 0.01 and 0.1 mg. of OT. Of the 122 persons in the category of repeated vaccinations, 103 (84.4 per cent) gave a severe inflammatory reaction following the injection of BCG vaccine although only one of this group of 103 persons had given a definite reaction to 0.1 mg. of OT. The intracutaneous injection of BCG vaccine in 103 persons in this category was followed 48 hours later by a diffuse deep red, slightly elevated, edematous area, painful to the touch but with no associated involvement of the regional axillary nodes. Thirteen persons in this category of repeated vaccinations showed a moderate post-vaccinal inflammatory reaction; in 4 persons the reaction was slight, and 2 persons were not examined.

The local ulcers of the 103 persons who developed a Koch phenomenon differed significantly from the ulcers of those who did not show a Koch phenomenon. When the ulcers of those who had shown a Koch phenomenon were observed 36 days after vaccination, the skin surrounding the ulcer for a distance of 10 to 20 mm. was dark red or cyanotic in appearance while the underlying subcutaneous tissue was indurated. The ulcers themselves, which had a punched-out appearance, were deeper and drier than those noted among persons receiving their initial vaccination. When observed 57 and 71 days after vaccination, they presented much the same appearance as they did 36 days after vaccination. In general the ulcers healed slowly and many were covered with a thick laminated scab of oyster shell-like appearance. In a few instances the ulcers had healed 71 days after vaccination. After 85 days many of the ulcers had healed, leaving a well-marked purplish, slightly depressed scar; and after 113 days, when the observations were discontinued, with but few exceptions the ulcers had healed.

The rate of healing and the appearance of the ulcer in the category of repeated vaccinations were comparable among the controls and in the isoniazid-treated persons.

SUMMARY

The effect of treatment with isoniazid on the tuberculin reaction and on the healing of ulcers following BCG vaccination in two groups of persons was studied. One group of 116 persons received their initial vaccination while the second group of 122 persons represented those who had been vaccinated from one to five times previously with BCG vaccine and who were again injected with BCG vaccine at the same time as the first group.

Six weeks after vaccination a significantly higher percentage of those who had received their initial vaccination reacted to 0.01 mg. of OT than did those who had previously received repeated injections of BCG vaccine.

Beginning 47 days after vaccination, approximately one half of those who had received an initial vaccination and one half who had received repeated vaccinations were given 5 mg. of isoniazid per kg. of body weight daily for 64 days, while the remaining half of each group served as controls.

Of those who had received their initial vaccination 111 days previously, a somewhat smaller but comparable percentage of both isoniazid-treated and control subjects reacted to 0.01 mg. of OT and to 0.1 mg. of OT than reacted 42 days after vaccination. Among those who had previously received repeated vaccinations, a larger but not statistically significant percentage of those treated with isoniazid failed to react to 0.01 mg. of OT after treatment than before treatment, while the percentage of control subjects who reacted to this dose of OT remained the same. The percentage who reacted to 0.1 mg. of OT, 42 and 111 days after vaccination, was approximately the same in the control and treated groups.

The character and rate of healing of the BCG-induced ulcers were not significantly different in the isoniazid-treated group when compared with the control group.

SUMARIO

Los Efectos de la Isoniacidoterapia sobre la Reacción a la Tuberculina y sobre la Cicatrización de las Ulceras Inducidas por la Vacuna BCG

Versó este estudio sobre el efecto del tratamiento con isoniacida sobre la reacción tuberculínica y sobre la cicatrización de las úlceras consecutivas a la vacunación con BCG en dos grupos de personas. Un grupo de 116 personas recibió su vacunación inicial, mientras que el segundo grupo de 122 personas representó a las vacunadas de uno a cinco años antes con BCG y a las que se inyectó de nuevo la vacuna al mismo tiempo que al primer grupo.

A las seis semanas de la vacunación un porcentaje significativamente más alto de los que habían recibido su vacunación inicial reaccionó a 0.01 mg. de TA que el de los que habían recibido inyecciones repetidas de vacuna BCG.

Partiendo de 47 días después de la vacunación, aproximadamente a la mitad de los que habían recibido una vacunación inicial y a la mitad de los que habían recibido revacunaciones se les administraron 5 mg. de isonacida por kg. de peso diariamente durante 64 días, en tanto que la mitad restante de cada grupo servía de testigo.

De los que habían recibido su vacunación inicial 111 días antes, un porcentaje algo menor, pero comparable, tanto de los tratados con isoniacida como de los testigos, reaccionó a 0.01 mg. de TA y a 0.1 mg. de TA que el que reaccionó a los 42 días de la vacunación. Entre los que habían recibido revacunaciones, un porcentaje mayor, pero sin importancia estadística, de los tratados con isoniacida no reaccionó a 0.01 mg. de TA después del tratamiento que antes de éste, mientras que el porcentaje de los testigos que reaccionaron a esta dosis de TA permaneció igual. El porcentaje de los que reaccionaron a 0.1 mg. de TA a los 42 y los 111 días de la vacunación fué aproximadamente idéntico en los grupos de testigos y tratados.

La naturaleza y la velocidad de la cicatrización de las úlceras provocadas por BCG no fueron significativamente distintas en el grupo tratado con isoniacida, comparado con el testigo.

RESUME

Les effets du traitement à l'isoniazide sur la réaction à la tuberculine et sur la cicatrisation des ulcérations engendrées par le vaccin BCG

L'effet de l'isoniazido-thérapie sur la réaction à la tuberculine et sur la cicatrisation des ulcérations après vaccination par le BCG a été étudié dans deux groupes de sujets. Un

groupe de 116 personnes recevait la vaccination initiale tandis que le second groupe de 122 personnes était constitué par ceux qui avaient été ultérieurement vaccinés de une à cinq fois avec le BCG et qui reçurent une nouvelle inoculation de BCG en même temps que le premier groupe.

Six semaines après la vaccination, le pourcentage qui réagissait à 0,01 mg de vieille tuberculine (OT) était significativement plus élevé chez ceux qui avaient reçu la vaccination initiale que dans le groupe ayant reçu des injections répétées de vaccin BCG.

A partir de 47 jours après la vaccination, approximativement la moitié de ceux ayant subi la vaccination initiale et la moitié de ceux ayant été soumis aux vaccinations répétées recevaient une dose d'isoniazide de 5 mg/kg chaque jour pendant 64 jours, tandis que la deuxième moitié de chaque groupe servait de témoins.

Parmi ceux qui avaient reçu leur vaccination initiale 111 jours avant, le pourcentage des sujets traités par l'isoniazide et des sujets du groupe-témoin qui réagissait à 0,01 mg de vieille tuberculine et à 0,1 mg de vieille tuberculine était un peu plus faible, mais comparable au pourcentage obtenu chez les sujets vaccinés depuis 42 jours.

Dans le groupe soumis aux vaccinations répétées, il a été obtenu, chez les individus traités par l'isoniazide, un pourcentage plus élevé, bien que non significatif statistiquement, de sujets qui ne réagissait pas à 0,01 mg de vieille tuberculine après traitement qu'avant traitement, alors que le pourcentage de sujets—témoins réagissant à cette dose de vieille tuberculine demeurait le même. Le pourcentage de réactions positives à 0,1 mg de vieille tuberculine, 42 et 115 jours après vaccination, était sensiblement le même pour le groupe traité et le groupe-témoin.

Le caractère et la vitesse de la cicatrisation des ulcérations engendrées par le BCG ne différaient pas sensiblement dans le groupe traité par l'isoniazide par comparaison avec le groupe-témoin.

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SERUM GAMMA GLOBULIN IN CHILDHOOD TUBERCULOSIS[1, 2]

CHARLOTTE MARKER ZITRIN, EDITH M. LINCOLN, ABRAHAM SAIFER, AND STEPHANIE LEWKOWICZ

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INTRODUCTION

Since the advent of gamma globulin as an entity, considerable interest has been centered on it as a diagnostic aid as well as a therapeutic agent (1, 2). Electrophoretic studies of serum proteins by Seibert and associates (3) showed a statistically significant rise in gamma globulin in minimal active tuberculosis which increased in proportion to the increasing severity of the disease. These findings have been corroborated by other investigators (4–8).

In 1952, Saifer and co-workers (9, 10) described in detail a new chemical method for detecting serum gamma globulin. The results compared favorably with the results obtained by electrophoresis, and the method was less time consuming and simpler to perform. Using this method in a clinical study, Volk and co-workers (4) corroborated the previous electrophoretic evidence that gamma globulin increased with the severity of tuberculous disease.

In a study of the gamma globulin levels in children, Lubschez (11) surveyed thirty normal children between the ages of two and eleven years. She found, by means of electrophoretic studies, that the gamma globulin values are slightly lower in the two- to four-year age group than in the older children. The values for the latter group were in close agreement with adult values, although there appeared to be greater individual variation. Lubschez also found that, in children who had an antecedent respiratory infection, an elevated gamma globulin followed in some cases. In a group of twenty-seven children, eleven were found to have abnormal gamma globulin values less than a month after infection and one child had an elevated gamma globulin one month following illness. A small number of determinations made after two and three months showed no abnormal gamma globulin levels at these times.

Knapp and Routh (12) also studied the electrophoretic patterns of plasma proteins in normal children and found that the gamma globulin was unusually high in the newborn, decreased to less than 0.5 gm. per 100 ml. in normal infants beyond the newborn period but less than one year of age, and rose but remained less than 1.0 gm. per 100 ml. in children one to four years of age. Levin and associates (13) studied gamma globulin in children with tuberculosis. They found that the gamma globulin rose early in the course of the disease and, in

[1] From the Department of Pediatrics, New York University College of Medicine and Chest Clinic of the Children's Medical Service, Bellevue Hospital Center, New York, New York; and the Isaac Albert Research Institute, Jewish Chronic Disease Hospital, Brooklyn, New York.

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general, paralleled the course of the erythrocyte sedimentation rate. However, in cases of tuberculous meningitis, they found a "gamma globulin—sedimentation rate dissociation" just before the onset of meningitis and just before death, with the gamma globulin falling rapidly and the sedimentation rate rising.

The principal purposes of the present study were to correlate the gamma globulin in cases of childhood tuberculosis with the clinical findings, and to compare two chemical methods for obtaining gamma globulin values in order to determine the simplest one for accurate correlation with clinical status. Another purpose of the study was to determine whether respiratory infections affect the gamma globulin levels sufficiently to interfere with its usefulness as a diagnostic and prognostic aid in children with tuberculosis.

MATERIALS AND METHODS

Eighteen children with various forms of active tuberculosis, on the wards of the Children's Medical Service of the Bellevue Hospital Center, were the subjects. The ages of the patients ranged from three months to twelve and one-half years. Blood was drawn at monthly intervals for the determination of albumin, total globulin, gamma globulin, thymol turbidity, and erythrocyte sedimentation rate. At approximately the same time, the child was given a physical examination, and a chest roentgenogram was obtained. A complete blood count and urinalysis were also done.

Gamma globulin was determined by two methods: the colorimetric flocculation-ninhydrin procedure of Saifer and co-workers (9, 10, 14) and the zinc sulfate turbidity method of Kunkel (15). The results were compared for reliability and ease of performance.

The thymol turbidity test (16, 17) has been widely used as a liver function test. Maclagan (18) has pointed out that, in this test, "gamma globulin is the important fraction and beta globulin acts only by contributing phospholipid." In view of the dependence of this test in large measure on gamma globulin, it was decided to include thymol turbidity curves for their interest, since none of these children had any evidence of liver disease.

The erythrocyte sedimentation rate was determined by the method of Wintrobe and Landsberg (19). All of the chemical determinations were done on the same sample of blood.

The clinical evaluation of each patient was based upon physical examination, temperature, weight, and roentgenographic and laboratory findings. Each child was classified as 1, 2, or 3 depending on the extent of the roentgenographic and laboratory evidence of tuberculous infection and *A*, *B*, or *C* depending on the severity of the clinical manifestations of disease, including physical examination, symptomatology, weight, and temperature. An increase from 1 to 3 and from *A* to *C* represents increasing severity of disease.

RESULTS

Table 1 contains all of the pertinent clinical and laboratory data on the 18 children in this study. The charts show graphically, for each patient, the gamma globulin and thymol turbidity curves. Diagnoses, positive cultures for tubercle bacilli, upper respiratory infections, and other complications are indicated on each graph. Gamma globulin (GG) injections given for measles prophylaxis are also indicated. The antituberculous therapy received is charted below each graph.

The normal range of serum gamma globulin as determined by the flocculation-ninhydrin method was between 0.72 and 1.25 gm. per 100 ml., with a mean of 1.00 gm. per 100 ml. (10). The normal range in this laboratory for the zinc sulfate turbidity method is from 6 to 12 Kunkel units (15). In the

TABLE 1

PERTINENT CLINICAL AND LABORATORY FINDINGS IN THE EIGHTEEN CASES OF THE PRESENT SERIES

Patient	Age	Sex	National Origin or Race	Diagnosis	Month	Clinical Evaluation	Gamma Globulin: Zinc Sulfate (Kunkel units)	Gamma Globulin: Ninhydrin (gm. per 100 ml.)	Albumin Globulin	Total Protein	Thymol Turbidity	Erythrocyte Sedimentation Rate	Complications
No. 1, E.P.	7 mos.	M	Puerto Rican	Subsiding primary tuberculosis	1	2A	8.5	0.89	4.6/2.1	6.7	7.00	—	
					2	2A	6.8	—	4.1/2.2	6.3	5.30	1.5	Upper respiratory infection
					3	2A	8.9	0.76	4.6/2.5	7.1	4.90	3.0	
No. 2, J.S.	5½ mos.	M	Negro	Primary tuberculosis	1	2B	25.8	2.23	4.5/4.8	9.3	13.90	21.0	
					2	2A	16.5	1.61	3.7/2.3	6.0	9.60	15.0	
					3	2A	13.4	0.87	3.8/2.5	6.3	7.00	—	
					4	2A	10.2	1.27	5.1/2.8	7.9	5.10	3.0	
					5	2A	11.0	—	5.3/2.5	7.8	3.10	—	
No. 3, M.A.	19 mos.	F	Puerto Rican	Primary tuberculosis + bronchiectasis	1	3B	16.9	1.16	4.4/2.2	6.6	9.30	—	
					2	2B	13.6	0.78	4.3/2.0	6.3	9.40	10.0	Upper respiratory infection
					3	2B	14.1	0.84	4.4/2.1	6.5	5.30	32.0	Upper respiratory infection
					4	2B	11.1	0.92	4.7/2.0	6.7	7.40	31.0	Upper respiratory infection
					5	2B	12.3	1.20	4.7/2.3	7.0	7.10	8.0	
					6	2A	11.6	1.43	4.9/1.2	7.1	8.30	5.0	
					7	2A	12.7	1.67	3.8/2.3	6.1	7.90	0.0	
No. 4, R.F.	7½ yrs.	M	Puerto Rican	Primary tuberculosis of the skin	1	2A	7.8	1.24	4.5/2.4	6.9	1.90	13.0	
					2	1A	8.1	0.74	3.7/1.9	5.6	1.60	10.0	
					3	1A	8.8	0.72	4.0/1.8	5.8	0.67	—	
No. 5, W.B.	3 yrs.	M	Negro	Scrofula + ? tuberculous kidney; healed primary complex in lung	1	2A	14.0	2.01	4.1/3.1	7.2	2.80	38.0	Albuminuria, hematuria
					2	2A	9.1	1.90	3.9/3.0	6.9	2.10	—	Trace of albuminuria
					3	2A	10.3	1.65	4.2/2.4	6.6	2.50	5.0	10–12 erythrocytes/high power field 1–2 leukocytes/high power field
					4	2A	11.2	1.84	3.8/2.6	6.4	2.80	5.0	Urine negative
No. 6, N.N.	10 yrs.	F	Puerto Rican	Tuberculous pleurisy with effusion	1	1A	12.9	1.42	4.0/2.9	6.9	2.70	14.0	
					2	1A	13.7	—	4.2/2.9	7.1	2.60	—	
					3	1A	13.6	1.20	4.3/2.9	7.2	4.30	23.0	
					4	1A	14.9	1.24	4.0/2.1	6.1	2.00	15.0	
No. 7, A.C.	12½ yrs.	F	Chinese	Tuberculous cervical adenitis	1	2A	12.4	1.81	5.3/3.1	8.4	6.90	23.0	
					2	2A	11.9	1.05	4.9/2.8	7.7	6.60	10.0	
					3	1A	13.2	1.24	4.3/2.7	7.0	3.30	15.0	
				Chronic pulmonary tuberculosis	6	2A	13.6	1.84	4.1/2.0	6.1	4.00	15.0	
					7	2A	13.1	1.91	4.0/2.5	6.5	4.80	8.0	Upper respiratory infection
					8	2A	13.5	1.33	4.7/2.3	7.0	3.60	9.0	
No. 8, A.S.	8 yrs.	M	Negro	Chronic pulmonary tuberculosis with bronchogenic spread	1	3B	31.0	—	—	—	5.30	—	Bronchial asthma
					2	3B	30.9	2.44	3.6/5.2	8.8	8.80	32.0	Bronchial asthma
					3	3B	26.5	2.52	3.7/3.8	7.5	6.10	33.0	Bronchial asthma
					4	3B	21.5	2.38	4.5/3.0	7.5	6.90	22.0	Bronchial asthma
					5	3B	19.5	2.61	3.8/3.8	7.6	4.50	—	Bronchial asthma
					6	2B	18.2	2.50	4.1/2.7	6.8	3.00	—	Bronchial asthma
					7	2A	15.7	2.04	3.8/3.2	7.0	2.60	—	Bronchial asthma
					8	2A	14.2	1.81	4.9/2.9	7.8	0.16	22.0	Bronchial asthma
No. 9, P.G.	11 yrs.	F	Puerto Rican	Chronic pulmonary tuberculosis	1	2B	17.9	1.71	4.6/2.9	7.5	6.50	32.0	Tuberculous enteritis; Mazzini, 4+
					2	2B	19.0	1.67	4.0/3.7	7.7	6.10	32.0	Healing tuberculous enteritis
					3	2A	23.3	1.77	4.3/2.9	7.2	7.60	38.0	

TABLE 1.—*Continued*

Patient	Age	Sex	National Origin or Race	Diagnosis	Month	Clinical Evaluation	Gamma Globulin		Albumin Globulin	Total Protein	Thymol Turbidity	Erythrocyte Sedimentation Rate	Complications
							Zinc Sulfate	Ninhydrin					
							Kunkel units	*gm. per 100 ml.*					
No. 10, W.P.	1 yr.	M	White	Miliary tuberculosis	1	3C	8.4	1.20	4.7/2.7	7.4	11.90	32.0	
					2	3B	4.0	0.73	5.3/1.8	7.1	3.60	22.0	Upper respiratory infection
					3	3A	5.7	0.94	4.8/1.7	6.5	4.50	10.0	
					4	2A	4.3	0.68	4.7/1.7	6.4	5.30	—	
					5	2A	21.8	—	5.2/2.1	7.3	12.50	—	
					6	1A	2.3	0.74	5.2/1.5	6.7	3.60	5.0	
No. 11, C.M.	5 mos.	M	Puerto Rican	Miliary tuberculosis with bronchogenic spread	1	3C	18.7	1.62	4.1/3.0	7.1	12.30	—	
					2	3B	19.0	1.32	4.0/2.8	6.8	9.90	22.0	
					3	2B	13.4	1.13	4.3/2.6	7.9	5.80	4.0	
					4	2B	12.4	1.47	4.1/3.0	7.1	9.10	32.0	
					5	2A	10.7	0.96	4.5/2.0	6.5	6.80	10.0	
					6	2A	6.8	1.36	5.7/2.8	8.5	1.70	—	Measles
					7	2A	19.0	2.47	4.0/2.7	6.7	9.80	—	
No. 12, R.M.	11 mos.	M	Puerto Rican	Miliary tuberculosis	1	3C	21.6	1.98	4.4/4.2	8.6	11.60	—	
					2	3C	18.7	2.19	4.8/3.8	8.3	7.40	40.0	
					3	3B	20.2	2.02	4.7/2.8	7.5	8.90	10.0	
					4	2A	13.6	1.71	4.7/1.8	6.5	6.50	15.0	Upper respiratory infection
					5	2A	13.0	1.46	5.9/3.3	9.2	5.80	5.0	
No. 13, E.N.	10 mos.	F	Chinese	Miliary tuberculosis	1	3C	23.8	1.96	4.8/3.2	8.0	19.80	34.0	
					2	3B	31.3	3.98	4.6/5.3	9.9	19.60	15.0	
					3	2B	26.8	2.50	5.0/2.9	7.9	15.90	22.0	
					4	2A	16.0	2.18	4.5/2.8	7.3	6.80	10.0	
					5	2A	18.8	2.04	5.4/3.5	8.9	3.60	—	
					6	2A	18.2	1.98	5.2/2.2	7.4	7.80	—	
No. 14, D.M.	19 mos.	M	White	Miliary tuberculosis + Pott's disease + tuberculous cervical adenitis	1	3C	8.8	0.96	4.2/3.1	6.3	5.80	—	
					2	3C	8.9	0.92	4.8/1.6	6.4	6.60	—	
					3	3C	6.6	1.07	4.9/3.3	8.2	7.30	21.0	
					4	3C	5.0	0.99	4.6/1.8	6.4	4.30	10.0	
					5	3C	7.1	0.62	6.0/1.8	7.8	5.80	—	
					6	3C	5.0	0.76	4.7/1.5	6.2	3.30	8.0	
					8	2B	4.8	0.75	6.2/1.8	8.0	3.10	—	
No. 15, E.O.	2 yrs.	M	Puerto Rican	Tuberculous meningitis	1	3C	7.8	1.40	4.7/3.3	8.0	3.00	14.0	
					3	3C	5.6	0.65	3.6/1.7	5.3	2.60	—	Secondary purulent meningitis; died
No. 16, W.M.	4 yrs.	M	White	Tuberculous meningitis	1	3C	4.8	0.63	4.7/2.0	6.7	5.80	16.0	
					2	2B	11.5	—	4.2/2.5	6.7	6.10	1.0	Secondary purulent meningitis; died
					3	3C	10.1	0.95	4.2/3.4	7.6	12.70	—	
No. 17, A.M.	2 yrs.	M	Puerto Rican	Tuberculous meningitis	1	3C	5.6	1.20	3.9/2.0	5.9	3.50	17.0	
					2	2B	18.9	1.60	5.3/4.1	9.4	11.70	16.0	Upper respiratory infection
					3	2B	16.0	1.96	5.6/3.0	8.6	8.60	11.0	Herpes zoster
					4	2A	11.9	—	5.3/2.2	7.5	4.00		
No. 18, J.M.	2 yrs.	F	White	Tuberculous meningitis	1	3C	6.8	1.52	4.8/2.2	7.0	4.80	10.0	
					2	2B	11.7	1.81	4.8/3.5	7.3	5.20	10.0	
					3	2B	17.2	1.99	4.6/2.3	6.9	7.90	14.0	
					4	2A	16.5	1.47	5.2/2.4	7.6	8.90	6.0	
					5	2A	13.4	1.99	4.9/2.5	7.4	7.60	30.0	

table and figures, the gamma globulin determined by the zinc sulfate method is expressed in terms of Kunkel units (KU) and the gamma globulin done by the flocculation-ninhydrin procedure as gm. per 100 ml. The upper limit of normal for thymol turbidity is 5 (17). The upper limit of normal for the corrected erythrocyte sedimentation rate is 15 mm. per hour (19).

It may be seen in the figures that, in the cases of active pulmonary tuberculosis, whether primary or chronic (reinfection) pulmonary, the gamma globulin was initially elevated, gradually returning to normal with improvement of the

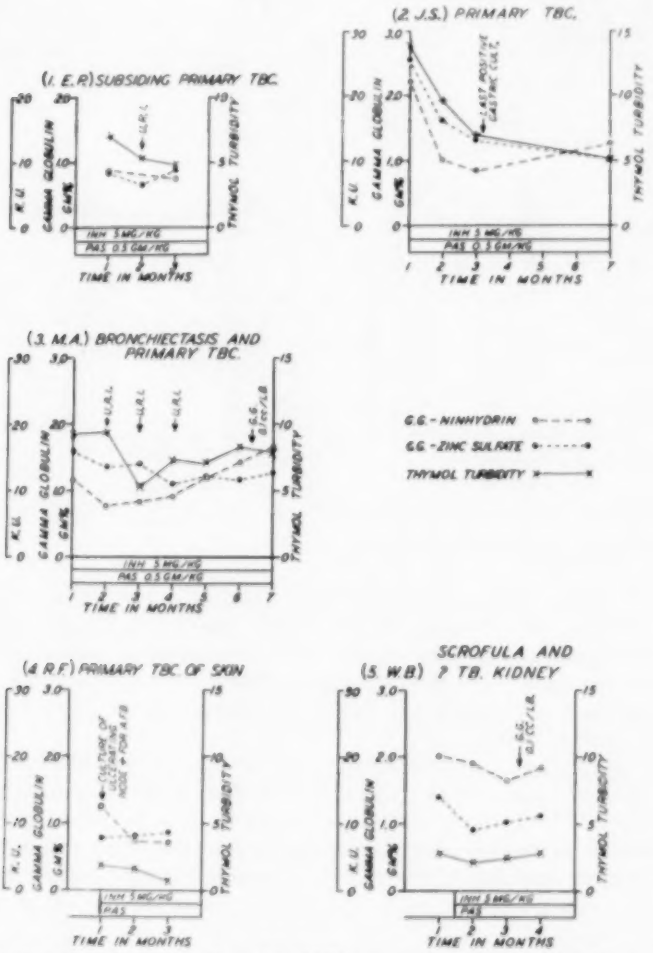

FIG. 1. Gamma globulin and thymol turbidity curves in patients with primary tuberculosis and scrofula.

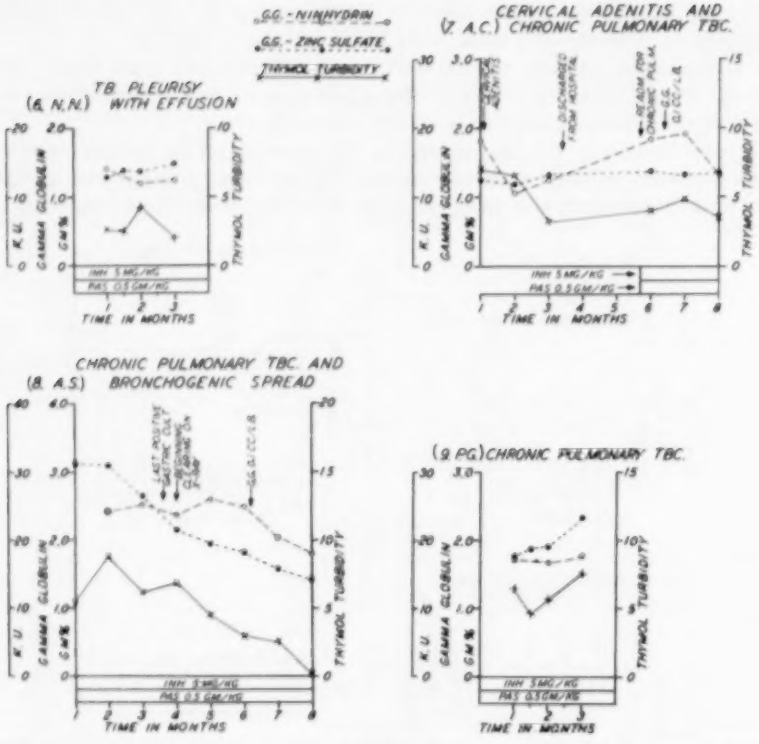

FIG. 2. Gamma globulin and thymol turbidity curves in patients with chronic pulmonary tuberculosis and tuberculous pleurisy.

patient. In miliary tuberculosis, 3 of the 5 patients showed initially elevated gamma globulin values and a gradually falling titer as the disease improved. These were patients who, clinically and by roentgenography showed early improvement (*see* table 1 and case summaries). The other 2 children with miliary tuberculosis had consistently low values for gamma globulin. These were the children who improved slowly.

Four of the patients had tuberculous meningitis. All of them had initially low gamma globulin values. In the 2 who died (*Nos. 15* and *16*), the gamma globulin remained low. The 2 who recovered showed an increase in gamma globulin to levels twice the normal mean as they improved clinically (*Nos. 17* and *18*).

Two children were found to have normal gamma globulin values. One had a healing pulmonary primary lesion, the other primary tuberculosis of the skin (*Nos. 1* and *4*).

In general, there was good correlation between both gamma globulin curves. It should also be noted that the thymol turbidity curves tended to parallel the

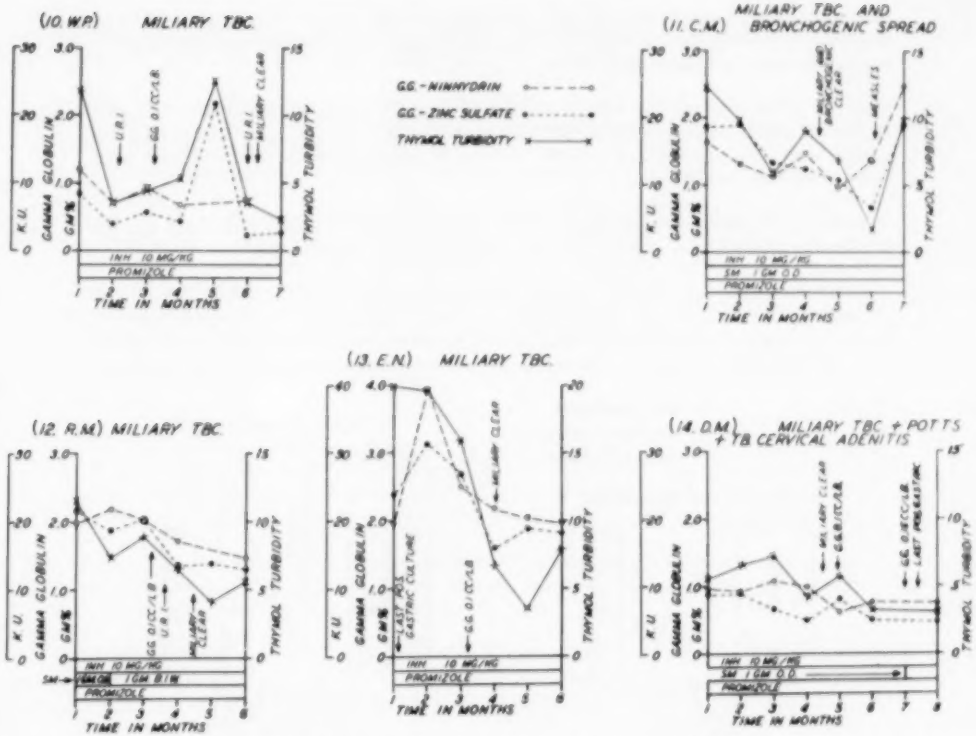

FIG. 3. Gamma globulin and thymol turbidity curves in patients with miliary tuberculosis.

gamma globulin curves. There was generally poor correspondence between clinical evaluation and the erythrocyte sedimentation rate. Upper respiratory infections (indicated in the figures by *U.R.I.*) did not appear to affect the gamma globulin level significantly.

Case Summaries

Case No. 1. A 7-month-old boy was treated for four months before the first blood determination. He showed gradual improvement and, at the time of the study, was clinically well and ready to be discharged from the hospital. All cultures for tubercle bacilli were negative.

Case No. 2 is that of a 3½-month-old boy who was in poor general condition on admission. He responded well to therapy and made an uneventful recovery.

Case No. 3 represented a 15-month-old girl who was acutely and chronically ill on admission. She responded well to antituberculous therapy which was started six weeks before the first blood determination. Cultures of gastric washings which were positive initially for tubercle bacilli were negative during the course of this study.

Case No. 4. A 7½-year-old boy was admitted nine weeks before the initial blood study with no pulmonary manifestations of tuberculosis and with gastric washings negative for tubercle bacilli. He made an uneventful recovery.

Case No. 5. A 3-year-old boy was admitted with scrofula which cleared quickly. Initially, he showed considerable hematuria and albuminuria; the urine gradually cleared and was normal after three and one-half months. Cultures of gastric washings and urine were negative for tubercle bacilli.

Case No. 6. A 10-year-old girl had a pleural effusion which cleared quickly and was not followed by any sequelae. All cultures were negative for tubercle bacilli.

Case No. 7. A 12½-year-old girl was hospitalized six months before the first blood determinations. At that time, she had tuberculous cervical adenitis for which she received no specific antimicrobial therapy. As the adenitis appeared to be subsiding, she was discharged from the hospital. Two and a half months later she returned to the hospital with a minimal

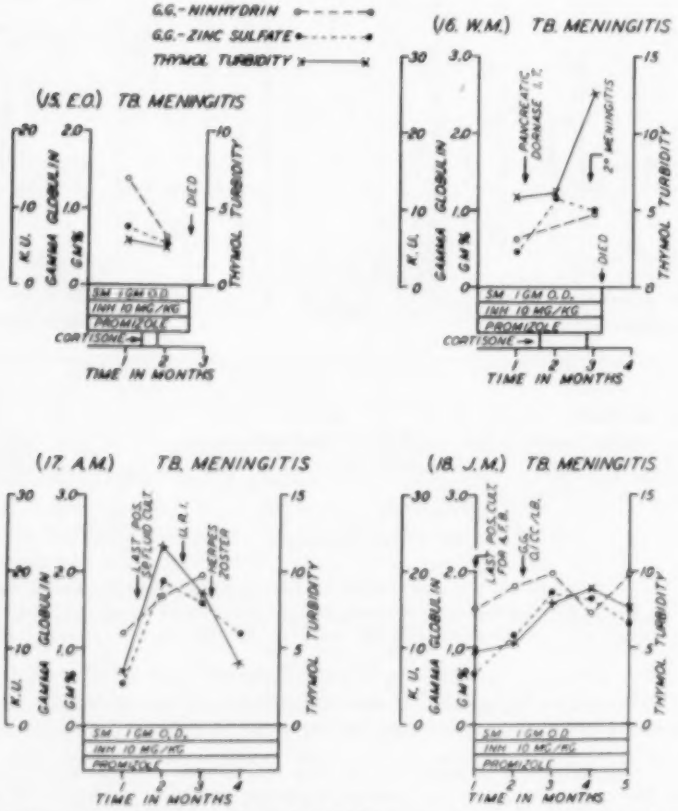

FIG. 4. Gamma globulin and thymol turbidity curves in patients with tuberculous meningitis.

lesion at the right apex. At this time, isoniazid and PAS were started and her lesion responded well. All cultures were negative for tubercle bacilli.

Case No. 8. An 8-year-old boy came into the hospital desperately ill, with moderately advanced chronic pulmonary tuberculosis and bronchogenic spread, superimposed on severe bronchial asthma. He responded well to therapy, becoming afebrile and gaining weight. Gastric cultures became negative for tubercle bacilli after three months of treatment.

Case No. 9. An 11-year-old girl came into the hospital six months before the first blood determination with far advanced chronic pulmonary tuberculosis and tuberculous enteritis. On admission she was severely malnourished and acutely and chronically ill. With antituberculous therapy, she improved considerably and, at the time of this study, she was gaining weight and had negative gastric cultures for tubercle bacilli. Roentgenographic examination, however, still showed the presence of bilateral apical disease.

The following are cases of miliary tuberculosis.

Case No. 10. The patient was a one-year-old boy who was pale, chronically ill, and febrile on admission to the hospital. He showed gradual but steady clinical improvement on antituberculous therapy. Nevertheless, his miliary lesions took twice as long (six months) to clear as the average case in this clinic. All cultures were negative for tubercle bacilli.

Case No. 11. A 5-month-old boy, who was acutely and chronically ill on admission, showed rapid clinical improvement with specific therapy and complete roentgenographic clearing of his miliary lesions in three months.

Case No. 12. An 11-month-old boy, acutely and chronically ill on admission, had complete roentgenographic clearing of the miliary lesions after four months of specific therapy and did well clinically. An initial gastric culture was positive for tubercle bacilli; subsequent cultures were negative.

Case No. 13. The patient was a 10-month-old girl who was malnourished and acutely and chronically ill on admission to the hospital. She made an uneventful recovery with complete clearing of miliary lesions in fourteen weeks. Two initial gastric cultures were positive for tubercle bacilli; subsequent cultures were negative.

Case No. 14. A 19-month-old boy was admitted to the hospital with miliary tuberculosis, primary pulmonary tuberculosis, tuberculosis of spine, ear, cervical nodes and, possibly, the kidney. Positive cultures for tubercle bacilli were obtained from ear, node, bronchoscopic secretions, and gastric washings. He was one of the most seriously ill patients and did not respond clinically, roentgenographically, or bacteriologically to isoniazid and Promizole® except for roentgenographic clearing of the miliary lesions in the lungs after three months. After five months of treatment, his infection was found to be partially resistant to isoniazid, and streptomycin was added. This was followed by disappearance of the tubercle bacilli from the various secretions and by clinical improvement.

The following are cases of tuberculous meningitis.

Case No. 15. A 2-year-old boy was seriously ill with tuberculous meningitis on admission to the hospital. In spite of intensive antituberculous therapy, his condition deteriorated and he developed a cerebrospinal block. Cortisone failed to benefit him and he died of secondary pyogenic meningitis.

Case No. 16. A 4-year-old boy who was critically ill on admission to the hospital was started on isoniazid, streptomycin, and Promizole. At first, he appeared to improve. However, he developed a cerebrospinal block and was treated first with pancreatic dornase, later with cortisone. Treatment failed to benefit him and he died of secondary pyogenic meningitis.

Case No. 17. A 2-year-old boy was acutely and chronically ill on admission to the hospital. His meningitis responded well to therapy, with "conversion" of cerebrospinal cultures to negative in three and a half weeks and progressive clinical improvement. He made an uneventful recovery.

Case No. 18. A 2-year-old girl was seriously ill with tuberculous meningitis on admission to the hospital. She responded rapidly to antituberculous therapy and made an uneventful recovery. The initial cerebrospinal fluid culture was the only one positive for tubercle bacilli.

DISCUSSION

The present study deals with too small a group of patients from which to draw any far-reaching conclusions. Nevertheless, it is believed that some interesting data have been obtained on these children and that some limited conclusions may be drawn.

In the children with active pulmonary tuberculosis, the gamma globulin levels were consistently elevated. As the children improved, the levels in 3 cases fell (*Nos. 2* and *8* by both methods; *No. 3* by the zinc sulfate method). In 2 patients there was no significant change, but they were followed only for two months (*Nos. 6* and *9*). A child with healing primary tuberculosis of the lung and one with primary tuberculosis of the skin had normal gamma globulin values (*Nos. 1* and *4*). A patient with scrofula (*No. 5*) showed a discrepancy between the two gamma globulin curves—the ninhydrin determinations were consistently elevated and the zinc sulfate determinations, which were initially elevated, later became normal. It should be pointed out that the clinical evaluations on each patient were made with no knowledge of the laboratory findings.

One child (*No. 7*) came into the hospital with cervical adenitis and, although she appeared to be doing well clinically, showed a slight rise in gamma globulin before discharge from the hospital. Two and a half months later, she was readmitted for a pulmonary lesion at the right apex. It was noted in retrospect that the lesion had been present at the time of the slight elevation of gamma globulin. By the time she was readmitted the gamma globulin, determined by the more sensitive ninhydrin method, had risen considerably (*see* table and figure 2). If the gamma globulin values had been followed during this interval, it is possible that the increasing titer might have called attention to the pulmonary lesion at an earlier time.

Of the children who had miliary tuberculosis, those who had initially elevated gamma globulin values were the ones who improved quickly, whereas those who had low gamma globulin values improved slowly. This raises some interesting questions. Is it possible to predict, with the aid of gamma globulin values, which children with miliary tuberculosis will handle their infections well, showing rapid improvement, and which have a relatively poorer prognosis? Perhaps

the high gamma globulin levels in the children who showed rapid improvement reflected a superior antibody production in those patients; perhaps those children whose gamma globulin levels were low had depressed antibody formation. Recent studies of agammaglobulinemia in adults with recurrent infections would tend to support this viewpoint (20, 21). If one carries this interpretation a step further, it would suggest that the latter group might be the one requiring more intensive antituberculous therapy, e.g., the simultaneous use of isoniazid, streptomycin, and Promizole, as well as adjunctive therapy, such as transfusions of blood or plasma or injections of gamma globulin, to obtain good clinical results.

As a case in point, one of these children (*No. 14*), who was treated with isoniazid and Promizole, began to discharge tubercle bacilli which were partially resistant to isoniazid. The patient failed to show clinical improvement until streptomycin was added to the therapeutic regimen. If such an unfavorable course could be predicted at an earlier date, it might be possible to prevent the emergence of drug-resistant tubercle bacilli and to shorten the course of the disease either by adjusting the therapy more rapidly or by treating it more intensively from the outset.

All 4 of the children with tuberculous meningitis had initially low values for gamma globulin. In the 2 who died the gamma globulin remained low, whereas in the 2 who recovered there were sharp rises in the gamma globulin values to considerably above the normal as the patient's condition improved. Here again, the evidence suggested that the initially low gamma globulin may be due to the inability of the body to mobilize its defenses for the production of antibodies in the presence of overwhelming infection. It is believed that children who survived were able to overcome this initially depressed antibody formation.

It is interesting that gamma globulin injections, given to these children for measles prevention, apparently had no effect on the gamma globulin. This finding would indicate that, should gamma globulin be considered for use as adjunctive therapy, large and, probably, repeated doses would be required, as in cases of agammaglobulinemia.

In 2 children who developed measles during the course of their tuberculous infection (one was *No. 11*, reported here; the other is to be reported in a later paper), the gamma globulin rose sharply following this complication. In a third child (*No. 10*), there was a similar rise at approximately the same time. However, this child had received prophylactic gamma globulin six weeks prior to the rise and showed no clinical signs of measles. Herpes zoster, on the other hand, appeared to have no effect on the gamma globulin curve (*No. 17*).

It must be borne in mind that laboratory tests of the type used in the present study do not have the precision of electrophoretic studies. Nevertheless, if the present tests are sufficiently accurate to aid the clinician in diagnosis and prognosis, the relative ease of performance of such tests as compared with electrophoresis must be weighted in their favor. It is believed that there is a fairly good correlation between the zinc sulfate and flocculation-ninhydrin methods of determining serum gamma globulin on the basis of the results of the present

study (table 1; figures 1–4). Since the former is considerably simpler to perform, it is adequate for clinical studies such as the present one, in which the trend of the curve in any one patient is the feature most helpful to the clinician.

In general, the thymol values corresponded fairly well to the gamma globulin values. This conforms with the general experience that, in patients in whom extrahepatic disease is the primary cause of changes in serum gamma globulin, the thymol turbidity may be deceptively low or high, as the case may be, and is not a valid liver function test.

In the past it has been generally believed on the Children's Chest Service at Bellevue Hospital that the erythrocyte sedimentation rate is not too helpful a diagnostic tool in primary tuberculosis as it may be affected by other factors, especially respiratory infections. In the present group of cases this impression has been confirmed, since there was no consistent correlation between the erythrocyte sedimentation rate and the clinical course of the tuberculosis.

The findings in this small group of children suggest that further investigation of serum gamma globulin levels in childhood tuberculosis may prove fruitful in helping to evaluate the clinical status of the child. The gamma globulin determinations may help one to decide whether the disease is quiescent or whether to expect an exacerbation; they may help in the prognosis of seriously ill patients with miliary tuberculosis or tuberculous meningitis and may point the way to therapy which might otherwise be delayed or omitted. The gamma globulin values will probably be most useful if serial determinations are made at, for example, monthly intervals. Then the trend of the curve in each patient may be evaluated in an attempt to add to the clinical knowledge of the progression or regression of the disease.

SUMMARY

Serum gamma globulin was found to be elevated in children with primary tuberculosis and chronic pulmonary (reinfection) tuberculosis.

In 4 cases of tuberculous meningitis, the initial gamma globulin values were low and in the 2 children who died the values remained low. In the 2 children who recovered, the gamma globulin values became moderately increased as the patients improved.

In the cases of miliary tuberculosis, 3 of 5 patients had initially elevated gamma globulin values. These were children who showed early improvement. The gamma globulin decreased gradually as the patients improved. The 2 remaining patients had initially low gamma globulin values which remained low for the entire course. These were the children who showed the slowest general improvement.

Upper respiratory infections and injections of gamma globulin for measles prophylaxis did not appear to affect the gamma globulin values in these patients.

The results of this study suggest that serum gamma globulin values in children with tuberculosis, particularly if serial determinations are made, may be of aid in the diagnosis and prognosis of this disease. The implications of this indication are discussed.

SUMARIO

La Seroglobulina Gamma en la Tuberculosis de la Niñez

La seroglobulina gamma se mostró elevada en los niños que tenían tuberculosis primaria y tuberculosis pulmonar crónica (reinfección).

En 4 casos de meningitis tuberculosa, los valores iniciales de globulina gamma eran bajos y, en 2 niños que fallecieron, permanecieron bajos. En los 2 que sanaron, las cifras de globulina gamma se elevaron moderadamente a medida que mejoraban los enfermos.

En los casos de granulia, 3 de 5 enfermos tenían al principio valores elevados de globulina gamma, siendo niños que revelaron mejoría temprana. La globulina disminuyó paulatinamente al mejorar los enfermos. Los 2 restantes tenían al principio valores bajos de la globulina, continuando así durante toda la evolución. Estos fueron los niños que mostraron la mejoría general más lenta.

Las infecciones de las vías aéreas superiores y las inyecciones de globulina gamma para la profilaxia del sarampión no parecieron afectar los valores de esta globulina en esos enfermos.

Sugieren los resultados de este estudio que los valores de seroglobulina gamma en los niños tuberculosos, en particular si se hacen determinaciones en serie, pueden resultar útiles en el diagnóstico y pronóstico de esta dolencia. Discútense los alcances de esta indicación.

RESUME

La globuline gamma du sérum dans la tuberculose de l'enfance

La globuline gamma sérique a été trouvée élevée chez les enfants atteints de tuberculose primaire et dans la tuberculose pulmonaire chronique (de réinfection).

Dans 4 cas de méningite tuberculeuse, les concentrations sériques initiales de globuline gamma étaient faibles et, chez 2 enfants qui ont succombé, ces concentrations demeuraient faibles. Chez les 2 enfants qui ont guéri les concentrations de globuline gamma ont augmenté de façon modérée et les malades se sont améliorés.

Dans les cas de tuberculose miliaire, il y avait chez 3 malades sur 5 initialement de hautes concentrations de globuline gamma. Il s'agissait d'enfants qui ont présenté une amélioration précoce. La globuline gamma a graduellement diminué à mesure que l'état des malades s'améliorait. Les 2 autres malades avaient initialement des concentrations de globuline gamma basses qui sont demeurées faibles pendant tout le cours de la maladie. C'est chez ces enfants que l'amélioration de l'état général a été la plus longue à se manifester.

Les infections des voies respiratoires supérieures et les injections de globuline gamma pour la prévention de la rougeole n'ont pas paru influencer la concentration de la globuline gamma chez ces malades.

Les résultats de cette étude suggèrent que les valeurs de la globuline gamma sérique chez les enfants atteints de tuberculose peuvent représenter une aide dans le diagnostic et le pronostic de cette maladie et en particulier lorsque les déterminations sont faites en série. Les implications de cette indication sont étudiées.

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PULMONARY RESECTION AS AN ADJUNCT IN THE TREATMENT OF PULMONARY TUBERCULOSIS[1, 2]

JOSEPH W. GALE, HELEN DICKIE, JOHN RANKIN, AND ANTHONY R. CURRERI

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INTRODUCTION

Until 1942, only 75 resections for pulmonary tuberculosis had been reported. With the development of better surgical techniques and the introduction of sulfonamides and penicillin, the use of pulmonary resection in tuberculosis continued. By 1947 numerous successful resections were reported by Janes (1), Maier and Klopstock (2), Overholt and associates (3), Sweet (4), and Bailey (5).

In 1944, after seventeen years of reasonably satisfactory experience with various surgical treatments for pulmonary tuberculosis, particularly thoracoplasty, the writers were aware of an ever increasing number of patients who had received collapse therapy, yet had active tuberculosis. Because surgical procedures in general use at that time failed to halt the discharge of tubercle bacilli in the sputum in a large proportion of patients, it was decided to change the surgical approach. It had always seemed to the writers that the removal of diseased tissue was the logical approach. With increased experience, it was found that many thoracoplasty failures were due partly to the lack of proper understanding of the natural history of certain types of the disease and partly to the choice of an inappropriate surgical procedure. It was believed that primarily surgery should assist the natural healing processes and, by removing residuals, diminish the opportunities for relapse.

The present report is concerned with pulmonary resection as an adjunct to the treatment of pulmonary tuberculosis. Consecutively operated cases from 1944 to May, 1954, are included. The results observed in the first 80 patients of the group were presented in 1949 (6). In the follow-up observations, it has been possible to obtain information on all but 50 of the 1,165 patients, or 95.7 per cent. When last heard from, the 50 patients on whom recent information is lacking were all well. All patients with granulomas of the lung without bacteriologic proof of tuberculosis have been eliminated from the series.

COMPOSITION OF SERIES

The extent of the pulmonary tuberculosis and the age of the patients are given in table 1. The largest group of patients had far advanced pulmonary tuberculosis. Only in the first three decades of life did the number of patients who had moderately advanced disease exceed those who had far advanced disease. One

[1] From the Departments of Surgery and Medicine, University of Wisconsin, Madison, Wisconsin.

[2] Presented before the Medical Session, as part of *Section 3A*, at the annual meeting of the National Tuberculosis Association, Milwaukee, Wisconsin, May 24, 1955.

TABLE 1

PULMONARY RESECTION

Classification of Disease

Age	Minimal	Moderate	Far Advanced
Years			
0–10	1	7	1
10–20	13	44	21
20–30	32	189	159
30–40	16	123	184
40–50	9	77	131
50–60	6	46	68
60–70	2	11	23
70–80		2	
Total (1,165)	79	499	587

TABLE 2

PULMONARY RESECTION

Types of Resection

Age	Segment	Lobe	Lung
Years			
0–10	4	5	
10–20	48	24	6
20–30	163	148	69
30–40	110	126	87
40–50	65	100	52
50–60	35	60	25
60–70	9	14	13
70–80		2	
Total (1,165)	434	479	252

hundred and fifty-eight of the 1,165 patients were fifty years of age or older at the time of their surgery. Thirty-eight patients were more than sixty years old.

The type of pulmonary resection in the various decades of life (table 2) followed a similar pattern in that patients more than thirty years old who had more extensive pulmonary disease likewise had more extensive resections. There was a sharp increase in segmental resections after 1950. In addition to the marked influence of long-term chemotherapy, the rise was also dependent on the decreasing number of thoracoplasty failures in the patient selected for resection. No primary collapse operations were performed after 1950, so that the decreasing number of thoracoplasty failures in this series is dependent primarily on the abandonment of this type of surgery rather than on the influence of long-term chemotherapy upon the success or failure of thoracoplasty.

METHODS

Antimicrobial Therapy

The use of streptomycin, para-aminosalicylic acid (PAS), and isoniazid has been so variable that tabulation is impossible. During the ten-year period under discussion, tremendous advances have taken place in the antimicrobial therapy of tuberculosis. Many facts have become established in regard to the clinical effect of more than a score of antituberculous drugs alone or in combination. These advances are reflected in the present series. From time to time, changes in therapy have been dictated as a result of the writers' experience or that of others.

At first streptomycin was used in large amounts without close surgical correlation. Soon it was recognized that the development of resistant strains of tubercle bacilli resulted in the loss of streptomycin protection, in itself a formidable threat to resection surgery. It was believed that early operation had to be instituted once streptomycin had been started. Later, when it was shown that a long-term, uninterrupted program of daily PAS and biweekly streptomycin offered a safe and effective method of treatment, the duration of preoperative therapy was lengthened considerably. The introduction of isoniazid in 1952 provided another potent antituberculous agent in the pre- and postoperative periods.

Under the tuberculostatic influence of long-term chemotherapy, surgical resection became possible at a better than reasonable cost in terms of operative mortality and postoperative complications. The general goal of drug therapy was the attainment of maximal resolution of reversible disease and the closure or eradication of open lesions. Persistent necrotic lesions which often harbored viable bacilli were then resected. In this way it was possible to restrict the extent of resections and preserve pulmonary tissue. The optimal time for surgical intervention varied considerably, depending on the extent and severity of the disease, the age of the patient, and the presence of complications such as endobronchitis. The average patient received nine to fifteen months of antimicrobial therapy prior to resection.

Bronchoscopy was performed several days before resection in nearly all patients. If any evidence of endobronchitis was present, surgical intervention was delayed. In some instances the addition of oxytetracycline to the antimicrobial therapy frequently cleared the minor endobronchitis which was probably nontuberculous.

The greatest therapeutic challenge, however, was the patient with persistent cavitation and endobronchitis whose tubercle bacilli had lost susceptibility to streptomycin and isoniazid. Pulmonary resection in these patients was hazardous because of the greatly increased risk of bronchopleural fistula. The only effective method of handling this problem is one of prevention of the loss of drug susceptibility. At the present time the tendency is to resect a cavitary lesion at an earlier period rather than to wait for reversal of infectiousness and cavitary closure to occur if this has not been accomplished after a four- to six-month period of treatment. If either streptomycin or isoniazid has not been used, it is added to the

drug regimen. At this time the advisability of surgical intervention is reviewed carefully. If surgery is regarded as feasible, the resection is planned within the four- to six-week interval after the addition of the streptomycin or the isoniazid. However, if, in spite of roentgenographic evidence of residual cavitation, the sputum no longer contains tubercle bacilli on microscopy or culture, the period of preoperative chemotherapy may be extended provided that by doing so the extent of removable pulmonary tissue can be significantly decreased. In the absence of cavitation and endobronchitis and when sputum "conversion" has been prompt, the improvement in the pulmonary tuberculous disease may continue for months. In such patients the timing of surgery is not critical. There is little reason to delay resection after maximum resolution of the reversible disease has occurred. The duration of the preoperative therapy in most patients will depend on the extent and character of the original disease.

In the immediate postoperative period (usually about five days), penicillin and streptomycin were administered twice daily. The duration of postoperative antituberculous chemotherapy varied considerably in the present series, depending on the extent of the unresected residuals. Chemotherapy was never given for less than six months during the period in which long-term uninterrupted treatment had been used.

SURGICAL TECHNIQUE

The surgical approach has varied in this group of patients but, as time has passed, a routine surgical technique has been adopted. All patients receive a careful bronchoscopic examination before operation. No patient is accepted for resection if an active tuberculous endobronchitis is present. A posterolateral approach is used and the sixth rib is resected throughout its length. Frequently the fifth and seventh ribs are divided just anterior to the transverse processes to provide more adequate exposure of the underlying lung. Adhesions are usually present near the apex or associated with the maximal disease. It has been found through bitter experience that, if the adhesions are dense, an extrapleural dissection is not only less shocking but also very easy and less apt to be complicated by postoperative bleeding.

As soon as the diseased lung is freed of offending adhesions, the hilum is exposed. If the right or left pulmonary artery is to be ligated, it is carefully isolated, then ligated with zero chromic catgut, and transfixed with cotton. Heavy catgut is used to prevent cutting through the wall of the pulmonary artery. This is particularly important in older patients with fragile arterial walls. The arteries are always ligated first to ensure emptying of the lung or lobe, thereby preserving blood which otherwise would be removed with the specimen. The bronchus is divided as close to its mother bronchus as possible. The writers use a Blalock clamp for this purpose. Division of the bronchus is made approximately 2 mm. distal to the clamp. It is then sutured with interrupted zero cotton sutures placed at 2 mm. intervals. Just proximal to these end sutures, interrupted zero chromic catgut mattress sutures are placed transversely across the bronchus. They are called "bumpers" and help to protect the end closure during the first few postoperative days when cough must be induced to keep the bronchi clear of secretions. On the right side, the main stem bronchus is always pleuralized after closure. On the left side, when divided close to the trachea, the stump retracts high above the inferior arch of the aorta into the areolar tissue and pleuralization is not necessary.

Care is always taken to isolate and ligate the bronchial artery. Experience has shown that it is a very free "bleeder," rivaled only by the artery in the pulmonary ligament.

Once hemastasis is established, 1 gm. of streptomycin and 200,000 units of penicillin are

placed in the pleural cavity. A one-half inch plastic tube is inserted through the chest wall at the costophrenic sinus in the posterolateral position. Suction drainage, varying between 2.5 and 4.0 cm. of water, is applied. Suction is maintained until any leak ceases and the lung is expanded. This usually occurs within forty-eight to seventy-two hours, but the tube may be left longer when air leaks persist—something most common following segmental resections. The patient is usually out of bed on the first postoperative day. It is believed that excessive tube suction is to be particularly avoided in the case of air leak or in atelectasis. Water-seal drainage has not been adequate in the writers' experience. If pneumonectomy is performed, usually no drainage is instituted, but during the first three days intrapleural pressures are carefully adjusted and the excess fluid is aspirated to minimize the chances of infection. Once the pleural surface regenerates, there is much less chance of infection by tubercle bacilli or other microorganisms. The ultimate success or failure of each resection for tuberculosis rests with careful and painstaking attention to the many small details.

RESULTS

There were thirty-six operative deaths in the present series of 1,165 patients with pulmonary tuberculosis. Twenty-one patients died following pneumonectomy, 13 after lobectomy, and 2 following segmental resection. The most common causes of death (table 3) were bronchopleural fistulas and intrapleural infection, pneumonitis on the same or contralateral side, respiratory failure, and atelec-

TABLE 3

PULMONARY RESECTION

Operative Deaths

Cause	Pneumonectomy	Lobectomy	Segmental
Bronchopleural fistula and sepsis	6	3	0
Pneumonitis, same side	0	1	0
Pneumonitis, opposite side	5	1	0
Respiratory failure, atelectasis, et cetera	3	1	1
Total	14	6	1

Miscellaneous Operative Deaths

Following pneumonectomy: 1 cerebral hemorrhage, 1 acute spread, 1 hemorrhage from pulmonary artery (ligature slipped), 1 pulmonary edema, 1 transfusion (overloading), 1 rupture of aorta (tuberculous aortitis), and 1 aspiration of blood following nasal hemorrhage. Total: 7

Following lobectomy: 1 cardiac failure and sepsis, 1 pulmonary artery thrombosis, 1 cardiac arrest, 1 sudden cause ? (no autopsy), 1 transfusion reaction, 1 contralateral pneumothorax, and 1 pulmonary embolus. Total: 7

Following segmental resection: 1 air embolus during transfusion. Total: 1

Total Operative Deaths

Following pneumonectomy	21
Following lobectomy	13
Following segmental resection	2
Total	36

TABLE 4

PULMONARY RESECTION

Summary of Bronchopleural Fistula

	Failed to Close		Dead
A. No special treatment	12	1	3
B. Closed by thoracotomy	8	3 (1 closed later)	0
C. Thoracotomy and closure	16	3 (2 closed later)	4
D. Open drainage only	3	1	1
Total	39 (3.3%)	8 − 3 = 5 (12.8%)	8 (20.5%)

tasis. The other causes of death which occurred are listed in table 3. All fatal cases in which the death could even remotely be associated with the surgical procedure have been listed as operative deaths.

Bronchopleural fistula, always associated with some degree of intrapleural infection, was the most frequent cause of death. The development of such fistulas can be attributed to the division of a bronchus which contains an active tuberculous endobronchitis, air leaks due to improper closure of a bronchus, and failure of antimicrobial therapy to control secondary lesions arising from spillage at the time of surgery.

Bronchopleural fistulas have been classified as (*a*) fistulas which received no special treatment, (*b*) fistulas which were closed successfully by thoracotomy, (*c*) fistulas which required thoracoplasty for closure, and (*d*) the group of fistulas in which open drainage of the pleural space was the only treatment necessary. The individual occurrence of these various forms of bronchopleural fistulas is presented in table 4. Among the 1,165 patients, bronchopleural fistulas developed in 39 patients (3.3 per cent); in 5 of the patients with fistulas (12.8 per cent), the opening persisted; and in 8 other patients (20.5 per cent) death occurred.

NONOPERATIVE DEATHS

Thirty-nine patients have died, and their deaths were not directly attributable to the surgical procedure (table 5). Eight patients died from active tuberculosis. Eight deaths have been classified as resulting from cor pulmonale. In this group, tuberculosis, although inactive at the time of death, must be charged with the responsibility for the chronic pulmonary insufficiency. The role that surgical intervention played in contributing to these deaths is impossible to evaluate. The one death from tracheal stenosis likewise was caused by the original tuberculous tracheitis although at necropsy the tuberculous background was not evident. The influence of the original tuberculous disease on the other tabulated causes of death is much less evident and in the majority it was probably not a contributory factor.

Bilateral resection was carried out in 96 patients (table 6). There were 3 deaths, a mortality of 3.1 per cent.

Ivalon® sponges were used as space fillers in 77 cases (table 7). Complications were chiefly due to pyogenic and tuberculous infection.

Hemorrhage, a most troublesome complication, occurred in 1.6 per cent of the pulmonary resections in this series (table 8).

Thoracoplasty, to obliterate the residual pleural space, was utilized in 62 patients (table 9). All pneumonectomies were followed by a thoracoplasty unless the pleural space was adequately collapsed due to the previous thoracoplasty. From the discrepancy in the number of the space-filling operations and the number of pneumonectomies, it is apparent that many pneumonectomies were per-

TABLE 5

PULMONARY RESECTION

Nonoperative Deaths

Cases	
4	Coronary heart disease
8	Cor pulmonale
4	Malignancy
4	Hypertension and renal disease
4	Suicide or overdose of barbiturates
2	Diabetes, 1 with complications
2	Toxemia of pregnancy
3	1 unknown, 1 tracheal stenosis (inactive), and 1 accidental
8	Tuberculosis
Total 39	

TABLE 6

PULMONARY RESECTION

Bilateral Resections

96 cases 3 deaths Mortality 3.1%

TABLE 7

PULMONARY RESECTION

Ivalon® Sponge—77 Cases

After segmental resection	15
After lobectomy	47
After pneumonectomy	15

16 removed: 7 due to pyogenic infection; 2, bronchopleural fistula (nontuberculous); 2, tuberculous infection; and 5, sterile reaction from G-11 (a detergent).

TABLE 8

PULMONARY RESECTION

Complications (Hemorrhage)

After segmental resection	9 (1 death, air embolus)
After lobectomy	6
After pneumonectomy	4
Total	19 (1.6%)

TABLE 9

PULMONARY RESECTION

Obliterative Thoracoplasty

Following segmental resection	4
Following lobectomy	22
Following pneumonectomy	36
Total	62

formed after thoracoplasty failures. A similar, but much less marked discrepancy exists in the lobectomy group.

Before 1950 when long-term uninterrupted chemotherapy was beginning to be used, the greater number of upper lobe lobectomies were followed by limited thoracoplasty to decrease the pleural space to minimize the chance of either a residual pleural pocket or overdistention of the remaining lung. After long-term chemotherapy, the danger of pleural space infection with tubercle bacilli was greatly reduced. In addition, the fibrosis and retraction of the major disease which was resected had progressed to the point at which the additional overdistention of the residual lung required to fill the small pleural space defect was minimal.

Since 1950 the space-filling procedures have been used primarily when it was evident that the remaining lung would not fill the pleural space without marked mediastinal shift. The presence of a persistent pneumothorax after resectional surgery is not desirable. In the experience of the writers, it usually indicates the presence of a small bronchopleural fistula. The hazard of subsequent tuberculous or nontuberculous infection of persistent pleural spaces is great enough to justify their obliteration.

LATE REACTIVATIONS AND COMPLICATIONS

Seventy-two of the 1,165 patients (6.1 per cent) have had continued or recurrent activity of their tuberculous disease. In 8 patients the sputum continued to contain tubercle bacilli after the resectional surgery. All of these patients have had surgery within the past year and a half; in all cases, the infection was resistant to the major antituberculous drugs prior to surgery. Whether they will eventually attain sputum "conversion" is doubtful. Fourteen patients who had been well have experienced a reactivation of their disease and are currently receiving treatment. Five of these have undergone additional pulmonary resection. Of these 14 with late reactivations, it is anticipated that the majority will eventually be well. Thirty-two of the 72 patients who have experienced further difficulty have had their reactivation sufficiently long ago so that adequate treatment has been given them and their disease may again be considered inactive. Of these 32 patients, 11 have had additional pulmonary resection. This group includes several patients who had refused bilateral resection until the reactivation of the tuberculosis convinced the patient of the advisability of this procedure.

Seven of the 32 reactivations, now classified as inactive, had recurrent diffi-

culties with tuberculous endobronchitis of the resection stump. They have all attained a noninfectious status and are well. Ten patients, whose only evidence of continued or recurrent tuberculous activity was the occasional discharge of tubercle bacilli, are included in the 72 patients. Of these 10, 8 have had subsequent gastric and sputum cultures negative for tubercle bacilli so that they may be classified as inactive. In 2 other patients, the gastric cultures which revealed tubercle bacilli are too recent to determine whether or not they will require an extended period of antimicrobial therapy. Chest roentgenograms and other studies did not establish any recognized source of the tubercle bacilli in these 10 patients.

Among the 72 patients, a group of 6 who have experienced extrapulmonary tuberculosis are included. Two late tuberculous abscesses in the thoracotomy wound area have cleared promptly with adequate drainage and antimicrobial therapy. No infection of the pleural space was found in these two wound abscesses. Cervical tuberculous lymphadenitis accounted for two other extrapulmonary reactivations. One of these patients is now well, but the lymphadenitis in the other was not recognized until December, 1954, and he is still being treated. A questionable tuberculous pericarditis occurring six months after a left pneumonectomy has caused the patient no trouble since 1950. Pott's disease of the lower thoracic spine was treated with a spinal fusion, and the patient has returned to normal activity and has remained well since 1953.

Of the 72 patients who have had continued or recurrent tuberculous disease, 47 are now well. Twenty-five patients have active tuberculosis.

Nontuberculous complications of resectional surgery have been noted in 7 other patients. Four Ivalon sponges have been removed because of a nontuberculous infection. Two nontuberculous empyemas have required open drainage. One pleural space infection which complicated a spontaneous pneumothorax was controlled by chemotherapy and aspiration without surgical intervention.

DISCUSSION

Owing to the varying clinical picture, the chronic nature of the disease, and the tendency to relapse, assessment of the results obtained by any form of therapy is difficult. Not many years ago the primary goal of treatment was to save life. Under modern management this is expected in most instances, and the emphasis is now placed on the rehabilitation of the patient, mentally and physically, and also on the prevention of relapse in the future. The writers believe that this is most rapidly and effectively achieved by close medical and surgical team work in which a planned course of antimicrobial drugs is followed by resection of necrotic foci and residual cavities.

For many years the routine sanatorium treatment of minimal pulmonary tuberculosis had been considered satisfactory in the majority of patients, but careful long-term studies have demonstrated a discouragingly high relapse rate of more than 30 per cent. It will be some time before evidence is available concerning the effectiveness of potent antituberculous drugs in reducing this hazard.

Justification for the resection of residual foci is considerably strengthened by

Medlar's concept of the role of caseous foci in the reactivation of pulmonary tuberculosis. In the process of healing, considerable resolution or fibrosis may occur, but caseous lesions frequently remain. These represent foci of necrosis which have never liquefied and sloughed into a bronchus or filled-in cavities. They contain tubercle bacilli and are closely associated with open bronchi. If more than a few millimeters in diameter, they rarely heal completely. Although frequently they remain "closed," they may at any time liquefy and slough into an adjoining bronchus and provide the focus for an aspiration spread of tubercle bacilli into other pulmonary segments.

The routine resection of caseous foci has been criticized on the basis that after prolonged drug therapy, although tubercle bacilli can be demonstrated, they are frequently difficult to isolate by culture or animal inoculation. It may also be pointed out that similar difficulty is experienced with lesions that have not been influenced by drug therapy; yet it is well known that such lesions are commonly responsible for the reactivation of arrested disease. At any rate, from the practical point of view, it is impossible to determine the viability of organisms in closed lesions prior to resection. In general, the larger lesions or residues of previous cavities are more likely to contain potentially dangerous organisms but, on the other hand, small lesions not infrequently harbor virulent organisms even after prolonged courses of potent antituberculous drugs. Recognizing this difficulty, the writers are perhaps more liberal in the selection of cases than many other workers in the field. In view of the low mortality and morbidity rate, as well as the results of long-term follow-up, the present approach seems the correct one.

When significant residuals remain, it is believed that surgery is contraindicated only in the presence of tuberculous endobronchitis or disease so extensive that its removal would seriously impair pulmonary function. Yet, even in the latter instance, the patient is often benefited by the removal of cavity-bearing areas. In the selection of cases, considerable help may be obtained from fluoroscopy, planigraphy, bronchography, and bronchoscopy. Pulmonary function studies may aid considerably in demonstrating the nature of any pulmonary disability present; but, like the electrocardiogram they supplement, they can never replace careful clinical evaluation. In rare instances resection can improve an already compromised pulmonary status.

In view of the failure to control the disease in many instances, the writers have abandoned all forms of primary collapse therapy. Thoracoplasty has still many advocates. The writers believe, however, that at best thoracoplasty may avert the disease and leave the most dangerous lesions *in situ*. A patient who is a poor risk for resection is an equally poor risk for thoracoplasty. There may be instances, however, when thoracoplasty prior to a planned resection may be of some value in selected cases. Certainly, following resection, thoracoplasty is a valuable space-filling procedure.

In patients in whom pulmonary function is limited and the residual pleural space is chiefly in the upper anterior portion of the pleural cavity, the insertion of an extraperiosteal Ivalon sponge is a better method of obliterating the space than any rib-removing operation. If no communication with the lung paren-

chyma persists to permit the entrance of air about the sponge, the subsequent development of tuberculous or nontuberculous infection of the space has been exceedingly uncommon in this series. Bilateral pulmonary resections may create a necessity for bilateral space-filling procedures and, in these circumstances, the use of Ivalon sponges on one or both sides has been very satisfactory.

Bronchopleural fistula remains the most serious problem in pulmonary resection for tuberculosis. An active tuberculous endobronchitis is the major cause of this serious complication of surgery. At the present time the endobronchitis can be controlled in nearly all patients if the tubercle bacilli remain susceptible to the major antituberculous drugs. In any patient the loss of such protection must be prevented if at all possible. The avoidance of using streptomycin and isoniazid together in the therapy leaves one drug which is capable of giving the adequate control of an endobronchitis even though resistance may have developed to the other. With the addition of the second drug, either streptomycin or isoniazid, it is essential to review the problem very carefully to be sure that surgery is not overlooked in the only period when it may be carried out with reasonable safety to the patient.

The development of resistance to the added drug and recurrence of an active endobronchitis may occur with great rapidity in the patient who has a persistent discharge of tubercle bacilli from an open cavity. If this has occurred and an endobronchitis exists in a patient whose infection has become resistant to both isoniazid and streptomycin, the problem is a complicated one. Viomycin and possibly pyrazinamide may be used, but they are not so effective. If an open cavity remains, resection of the lobe or lung is performed with acceptance of the risk of this complication. If the fistula is recognized early and adequate drainage is established to prevent the "spill" to the contralateral lung, secondary closure and collapse measures may be done later with reasonable success. The writers are aware that they have not proved that surgical removal of the residual necrotic foci remaining after long-term antimicrobial therapy is necessary or advisable. This group of patients includes many whose disease antedated the present concepts of therapy. It also includes a reasonable number whose long-term antituberculous therapy failed to control their disease. It has been the policy to accept extremely poor-risk patients in an endeavor to control their disease as well as to attempt to assess the risk to such patients. In spite of this, the operative mortality for pulmonary resection has been 3.1 per cent. The writers believe that it is indicated that pulmonary resection can be performed at an operative risk which need not be a deterrent to the inclusion of pulmonary resection in the treatment of any type of pulmonary tuberculosis. This holds true only if this form of treatment is advisable either from the standpoint of furnishing additional information in properly controlled studies or if it is believed that chemotherapy alone will not be adequate to control the patient's disease.

SUMMARY

Pulmonary resection was used in 1,165 patients who had pulmonary tuberculosis during the ten-year period from 1944 to May, 1954 (table 10). Current information has been obtained on 1,115 of this group of patients who have been

TABLE 10

PULMONARY RESECTION

Summary of 1,165 Resections

	Number	Per Cent
Operative deaths	36	3.1
Nonoperative deaths	39	3.3
Patients with sputum now positive for tubercle bacilli	25	2.1
Patients with sputum now negative for tubercle bacilli	1,015	87.1*

* Figure based on number on which current information is available.

followed from one to ten years. The operative mortality was 3 per cent. An additional 3 per cent of the patients have died in the intervening years from various causes, including active pulmonary tuberculosis Two per cent of the patients still are classified as having active tuberculosis. Of the 1,115 patients on whom the writers have current information, 1,015 (91 per cent) are clinically well and are classified as having inactive pulmonary tuberculosis. If only the patients who are living and on whom information is available are considered, 97 per cent are now classified as having inactive pulmonary tuberculosis.

SUMARIO

La Resección Pulmonar como Coadyuvante en el Tratamiento de la Tuberculosis Pulmonar

En 1,165 tuberculosos pulmonares se usó la resección pulmonar durante el decenio comprendido de 1944 a mayo de 1954. Se ha obtenido información al día para 1,115 enfermos de este grupo que han sido mantenidos en observación de uno a diez años. La mortalidad operatoria fué de 3 por ciento. Otro 3 por ciento de los enfermos han fallecido en los años intermedios de varias causas, incluso tuberculosis pulmonar activa. Dos por ciento de los enfermos están todavía clasificados como padeciendo de tuberculosis activa. De los 1,115 enfermos acerca de los cuales hay información actual, 1,015 (91 por ciento) se hallan clínicamente bien y están clasificados como casos de tuberculosis pulmonar inactiva. Si sólo se considera a los sujetos vivos y sobre los cuales hay datos disponibles, 97 por ciento están hoy día clasificados como casos de tuberculosis pulmonar inactiva.

RESUME

La résection pulmonaire comme adjuvant au traitement de la tuberculose pulmonaire

La résection pulmonaire a été pratiquée chez 1.165 malades atteints de tuberculose pulmonaire pendant une période de dix années de 1944 à Mai 1954. Il a été obtenu des informations récentes sur 1.115 membres de ce groupe de malades qui ont été suivis pendant des périodes allant de un à dix ans. La mortalité opératoire a été de 3 pour 100. Les décès dus à diverses causes, y compris la tuberculose évolutive, représentent un pourcentage additionnel de 3 pour 100. Le pourcentage des malades classés dans la catégorie des cas encore en activité est de 2 pour 100. Parmi les 1.115 malades sur lesquels les auteurs possédent des données récentes, 1.015 (91%) sont en bon état clinique et classés dans la catégorie des cas de tuberculose pulmonaire stabilisée. Si l'on considère seulement les malades vivants sur lesquels on possède des informations 97 pour cent figurent maintenant dans la catégorie des cas de tuberculose pulmonaire stabilisée.

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THE EFFECTS OF SOME SULFHYDRYL COMPOUNDS ON GROWTH OF CATALASE-POSITIVE AND CATALASE-NEGATIVE TUBERCLE BACILLI[1,2,3]

C. M. COLEMAN[4] AND G. MIDDLEBROOK

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INTRODUCTION

The diminished pathogenicity of isoniazid-resistant mutants of tubercle bacilli has been correlated with their deficiency in catalase activity (1–4).

Experiments have already been described showing that such organisms as are completely deficient in catalase activity (catalase-negative) fail to grow under certain conditions *in vitro* in the absence of hydrogen peroxide-decomposing agents (1). It was suggested that similar conditions may exist in phagocytes in the tuberculous animal. However, catalase-diminished, but not completely catalase-deficient, isoniazid-resistant mutants were not seen to manifest any special growth requirements *in vitro*. Nevertheless, such strains have been shown to be slightly to moderately attenuated for guinea pigs (5), hamsters (6), and rhesus monkeys (7). No reasonable explanation has been available for the failure of such strains to be fully pathogenic.

During the course of investigation of the effects of sulfhydryl compounds on the growth of tubercle bacilli, some observations have been made which appear pertinent to the mechanism of attenuation of catalase-diminished as well as catalase-negative mutants of tubercle bacilli. It is the purpose of this paper to describe these observations.

MATERIALS AND METHODS

Strains: The stock laboratory strains of tubercle bacilli, H37Rv and Pearson, employed in these experiments, have already been described (1). The catalase-negative, isoniazid-resistant mutants of these stock laboratory strains were also used. The latter showed partial inhibition in the presence of 100 γ of isoniazid per ml. of liquid "Standard Transfer Medium." They were routinely carried in Standard Transfer Medium. This is identical with medium 7H4 (1) except that 0.01 mg. of ferric ammonium citrate per ml. and 0.2 per cent glycerol are present in Standard Transfer Medium while casein-hydrolysate is omitted. The Pearson strain, possessing low catalase activity with intermediate isoniazid resistance (Pearson II-B), was obtained as previously described (1) by cultivation of the parent, isoniazid-susceptible, Pearson I strain in low concentrations of isoniazid in the presence of manganous ions which specifically inhibit growth of catalase-negative mutant organisms.

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[1] From the Department of Research and Laboratories of the National Jewish Hospital at Denver, Colorado, and the Department of Microbiology of the University of Colorado School of Medicine, Denver, Colorado.

[2] Presented before the Medical Session, as part of *Section 3B*, at the annual meeting of the National Tuberculosis Association, Milwaukee, Wisconsin, May 24, 1955.

[3] Part of a thesis submitted to the University of Colorado in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[4] Fellow of the medical section of the National Tuberculosis Association.

The Pearson II-B strain was inhibited by 1 to 5 γ of isoniazid per ml. in Standard Transfer Medium, while Pearson I was inhibited by 0.04 γ per ml.

Culture media: The semi-solid synthetic medium was prepared by autoclaving the previously described "Special Medium II" (SM-II) (1) which contains asparagine, ammonium chloride, alkali phosphates, magnesium sulfate, sodium citrate, and traces of calcium, zinc, and copper ions, along with 0.5 per cent agar. After autoclaving, 0.5 per cent glucose[4] and 0.5 per cent Cohn's fraction V albumin[5] were routinely added to this medium; when catalase or sodium pyruvate was used, they were added aseptically to the medium, which was then dispensed into sterile, screw-capped tubes containing various concentrations of sulfhydryl compound.

Organic reagents: Organic reagents,[7] which included sodium pyruvate, glutathione, oxidized glutathione, cysteine, thioglycollic, thiomalic, and ascorbic acids, were used after having been freshly dissolved and autoclaved as 10 per cent (w/v) solutions. The reducing agents were not significantly oxidized when autoclaved in such high concentrations and were subsequently dispensed aseptically, as needed.

Catalase[8] was dissolved in neutral, phosphate-buffered saline, sterilized by filtration through sintered glass, and stored at 4°C. in a concentration of 1 mg. per ml. It maintained its activity without significant change for periods of several months under these conditions. It was always added aseptically to the culture media in which it was used. None of its activity was dialyzable.

Conditions of incubation: Cultures were incubated for growth at 37° to 38°C. for one week, exposed to atmospheric air. When cultures were incubated at reduced air pressure, the tubes were placed in a desiccator jar and the total atmospheric pressure was reduced to give the partial pressure of oxygen desired.

Inocula: Erlenmeyer flasks containing semi-solid SM-II medium were inoculated at approximately 42°C. with 1 per cent of a fully grown Standard Transfer Medium culture. The flasks were then mixed and dispensed into screw-capped 20 by 125 mm. tubes containing the appropriate amounts of sulfhydryl compound. Blowing the semi-solid agar mixture into the tubes ensured adequate mixing of the culture and the solution of sulfhydryl compound.

RESULTS

The minimum concentrations of glutathione for the inhibition of growth of the catalase-negative, isoniazid-resistant strains of H37Rv and Pearson in SM-II liquid medium were 0.0025 per cent and 0.003 per cent, respectively. On the other hand, 1 per cent and 0.5 per cent glutathione, respectively, were needed to inhibit their catalase-positive, isoniazid-susceptible parent strains. For the catalase-diminished, partially isoniazid-resistant Pearson strain, 0.01 per cent glutathione was required to inhibit growth.

Addition of 1 γ of catalase per ml. to the medium allowed growth of the isoniazid-resistant catalase-negative organisms to take place in the presence of as much as 0.04 per cent glutathione.

The isoniazid-resistant, catalase-diminished Pearson II-B strain has a relatively low catalase activity as measured by quantitative hydrogen peroxide

[4] Obtained from Merck & Co., as "Dextrose Anhydrous Reagent."

[5] Bovine serum albumin (Cohn's fraction V) obtained from Armour Laboratories, Chicago, Illinois; or from the Pentex Company, 660 North Schuyler Avenue, Kankakee, Illinois.

[7] Obtained from the Nutritional Biochemicals Corporation.

[8] Obtained as a dry powder from Nutritional Biochemicals Corporation under the label "Catalase (Lyophilized) Beef Liver, 40 to 50 milliequivalents of sodium pyruvate."

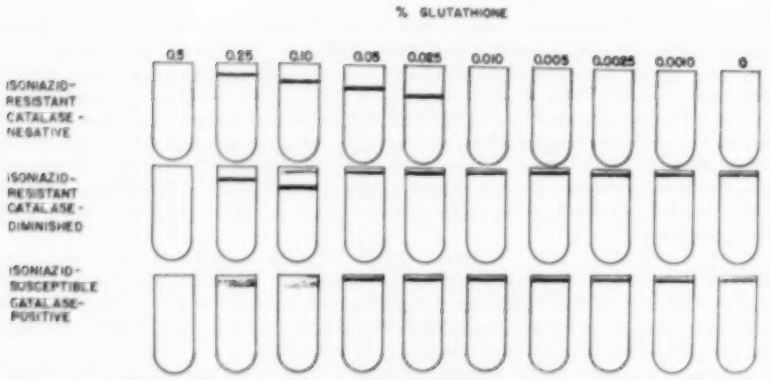

FIG. 1. Growth of Pearson strains in semi-solid medium containing various concentrations of glutathione. Agar plugs of semi-solid media are represented here with their bacterial growth in these schematic diagrams. The uppermost horizontal line in each figure represents the top edge of the agar plug while, below this, the thicker horizontal line or shaded area represents bacterial growth after seven days.

decomposition in the conventional Warburg manometer. It showed at the end of a three-hour operating period, using equi-dense suspensions at 0.5°C., a catalase activity approximately one-sixth that of the isoniazid-susceptible, catalase-positive parent strain. The isoniazid-resistant, catalase-negative strain showed no detectable catalase activity.

In figure 1 schematic diagrams are presented which show side views of the culture tubes containing various concentrations of glutathione (or thioglycollate). The organisms shown are the Pearson strains, but the H37Rv catalase-positive and -negative strains act similarly. There has been no successful isolation of an H37Rv isoniazid-resistant, catalase-diminished strain. The dark lines represent a side view of bacterial growth in the tubes. This occurred in discs perpendicular to the axis of the tube. The relative distance of the discs of growth of the isoniazid-resistant, catalase-negative organisms from the surface of the agar may be seen to be an inverse function of the concentration of added sulfhydryl compound. The isoniazid-susceptible, catalase-positive organisms may be seen to grow in discs near the surface of the agar up to the point where growth is inhibited by high concentrations of reducing agent. The intermediate isoniazid-resistant, catalase-diminished Pearson H-B strain gave an intermediate type of response to the glutathione. In general, similar results were also obtained with the sulfhydryl compounds, cysteine and thiomalate. Ascorbic acid, which also forms hydrogen peroxide on oxidation by oxygen, showed inhibition of growth, but did not give clear-cut rings of growth of the catalase-negative organisms. This is presumed to be due to the fact that it reacts much more rapidly with oxygen than glutathione and thioglycollic acid.

The addition of catalase or sodium pyruvate to the semi-solid medium containing the isoniazid-resistant, catalase-negative organisms permitted growth to occur

at the surface in the tubes containing no glutathione or low concentrations of glutathione, as shown in figure 2. Thus, catalase-negative tubercle bacilli behaved under these conditions as phenocopies of catalase-positive organisms when lower concentrations of glutathione were employed. When the concentration of catalase or sodium pyruvate was increased tenfold, the catalase-negative organisms were able to grow on the surface at the next higher concentration of glutathione. The possibility of genetic reverse mutation of the catalase-negative strains to the catalase-positive type was excluded: subcultures of the phenocopies were catalase negative.

When the catalase-negative organisms were incubated under low partial pressures of oxygen in different concentrations of thioglycollate, growth occurred closer to the surface, as seen in figure 3.

Concentrations of oxidized-glutathione up to 0.25 per cent were added to semi-solid SM-II medium, but caused no inhibition of growth of isoniazid-resistant, catalase-negative organisms.

In the presence of reduced glutathione, 0.4 γ $MnSO_4$-$4H_2O$ per ml. did not prevent the growth of isoniazid-resistant, catalase-negative organisms in semi-solid agar and liquid medium. $MnSO_4$-$4H_2O$ at this concentration has been previously shown to inhibit completely growth of catalase-negative tubercle bacilli if catalase or pyruvate are not present (1).

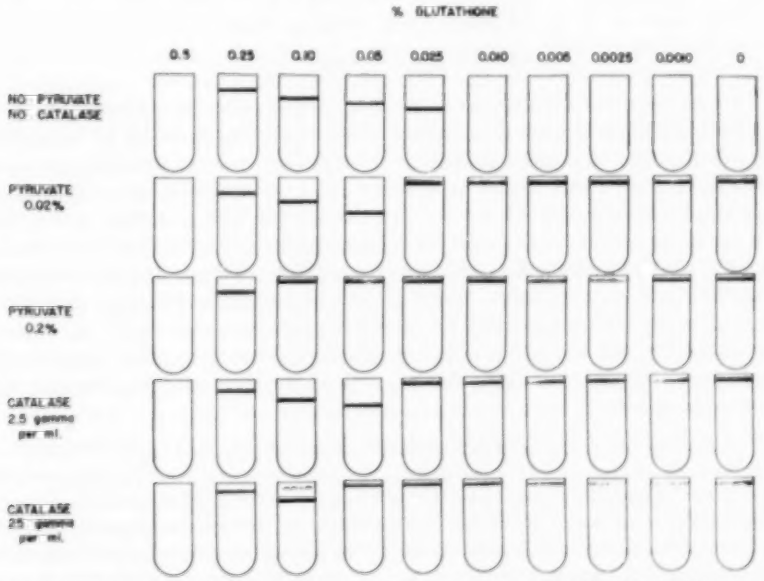

FIG. 2. Effect of catalase and sodium pyruvate on growth of H37Rv isoniazid-resistant catalase-negative strain in semi-solid media with glutathione. The agar plugs are represented here in the same manner as in figure 1. Growth is shown after seven days.

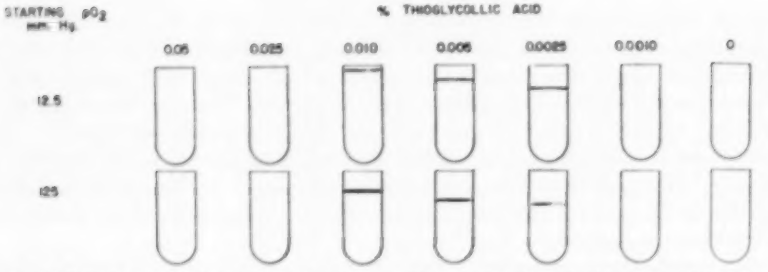

FIG. 3. Effect of partial pressure of oxygen on the growth of isoniazid-resistant catalase-negative H37Rv strain in semi-solid media containing thioglycollate. The agar plugs are represented here in the same manner as in figure 1. Growth is shown after seven days. The low partial pressure was attained by evacuation of the air from a desiccator jar containing the tubes of media.

DISCUSSION

Although several sulfhydryl compounds were tried, glutathione was most extensively used in these experiments since it is present in high concentrations in mammalian cells and is considered to be of prime importance in poising intracellular redox conditions.

The oxidation of glutathione is known to occur in the following manner (8):

$$2\,GSH + O_2 \rightarrow GSSG + H_2O_2$$

$$2\,GSH + H_2O_2 \rightarrow GSSG + 2H_2O$$

This oxidation of glutathione to yield hydrogen peroxide is considered to be the basis of the inhibition of growth of tubercle bacilli devoid of, or diminished in, catalase. In the present studies the narrow disc band of bacterial growth is apparently the critical border zone where both metabolically available oxygen and the electrode potential are at levels compatible with growth. Growth does not occur above the disc because the hydrogen peroxide produced there inhibits growth, while, below the disc, the reducing gradient becomes too high to permit growth. Divalent manganese, known to be uniquely toxic for catalase-negative organisms, unless an hydrogen peroxide-decomposing agent (such as catalase) is present (1), did not prevent the formation of these discs. This suggests that hydrogen peroxide is actively decomposed in the disc zone by reacting with sulfhydryl groups.

In summary, the following observations are consistent with the hypothesis that hydrogen peroxide derived from the continuous oxidation of glutathione by oxygen is the substance responsible for the growth inhibition of catalase-negative tubercle bacilli under these conditions: (*1*) catalase and pyruvate permit catalase-negative organisms to grow at the surface; (*2*) increasing the concentrations of these substances allows the catalase-negative organisms to grow on the surface at increasingly higher concentrations of glutathione; and (*3*) the cultures, when incubated at a low partial pressure of oxygen, which undoubtedly allows the glutathione to be oxidized more slowly, grew in discs closer to the surface of the agar.

Thus, the evidence seems conclusive that, under these conditions, hydrogen peroxide is the inhibitory substance.

Binet and associates (9), found the percentage of reduced glutathione in rat tissues as follows: liver 0.20, kidney 0.16, lung 0.075, and muscle 0.03.

Thus, concentrations of glutathione (or similar sulfhydryl compounds) probably occur intracellularly in that range which inhibits catalase-negative organisms under aerobic conditions, as shown by the above evidence. It has been observed that brucella (10), and plague bacilli (11), as well as tubercle bacilli, are attenuated when they are genetically devoid of catalase activity. It is suggested that such bacteria are attenuated because they are subjected, *in vivo*, to a minute steady-state concentration of hydrogen peroxide produced by the continuous oxidation of glutathione or of other hydrogen peroxide-yielding systems operating in phagocytic cells.

It might be objected that certain other pathogenic bacteria, such as pneumococci, streptococci, and some strains of anthrax bacilli (12) are pathogenic in spite of their deficiency in catalase. However, these parasites do not require an intracellular existence inside phagocytes for pathogenicity.

It has been observed that certain strains of tubercle bacilli possess a moderate degree of resistance to isoniazid along with a diminished catalase activity and only moderate pathogenicity. Such strains, in the present studies, also show an intermediate susceptibility to the toxic effects of hydrogen peroxide derived from glutathione oxidation. It is suggested that this reduction in pathogenicity may be a result of the intermediate susceptibility of such strains to hydrogen peroxide produced in host cells—perhaps by the oxidation of glutathione or of other reducing systems which produce hydrogen peroxide on oxidation by molecular oxygen, e.g., riboflavin oxidases.

It might be supposed that catalase, present in the host cell, could protect tubercle bacilli against intracellularly produced hydrogen peroxide; there is considerable evidence, however, that catalase is concentrated in intracellular particles and therefore may not be readily available for the protection of these parasites (13, 14). Moreover, it was seen in figure 2 that neither catalase nor pyruvate could completely counteract the action of hydrogen peroxide produced by the oxidation of glutathione *in vitro* under the experimental conditions described here. In other words, although minute concentrations of hydrogen peroxide-decomposing agents such as catalase are needed by catalase-negative organisms to decompose their own hydrogen peroxide, much higher concentrations of hydrogen peroxide-decomposing agents are required to protect catalase-deficient tubercle bacilli against exogenous hydrogen peroxide, as shown by these studies *in vitro*.

Actual production of hydrogen peroxide in mammalian tissues has not been detected. Its reactivity is such that the low concentrations which might be anticipated would not be directly measured by presently known methods. Nevertheless, its production and participation in the physiology of mammalian cells is strongly supported by the widely demonstrated presence of hydroperoxidases (catalase and various peroxidases) in such cells.

Catalase-negative tubercle bacilli multiply as freely as catalase-positive organisms for several days during primary infection. On the other hand, once the infection is established, catalase-negative organisms, in contrast to catalase-positive organisms, are unable to continue multiplication to produce disease (2, 15, 16). Therefore, if the hypothesis presented here is correct, the steady-state concentration of hydrogen peroxide, presumed to be in the cytoplasm of phagocytes which engulf tubercle bacilli in the animal with established tuberculosis, should be higher than that inside phagocytes of normal hosts during the first phase of the infection.

SUMMARY

Sulfhydryl compounds, glutathione in particular, either completely inhibit the growth of isoniazid-resistant, catalase-negative tubercle bacilli or only allow their growth to occur in discs beneath the surface of a semi-solid synthetic medium, depending on the concentration of the sulfhydryl compound.

Catalase and pyruvate antagonize these effects of glutathione on catalase-negative organisms in this medium and cause such organisms to behave as phenocopies of catalase-positive tubercle bacilli.

The antibacterial effects of glutathione under these conditions are attributed primarily to its ability to produce hydrogen peroxide on oxidation by molecular oxygen.

These observations provide a possible biochemical explanation for the decreased pathogenicity of catalase-diminished strains as well as catalase-negative strains in certain hosts.

SUMARIO

Los Efectos de Algunos Compuestos Sulfhidrílicos sobre la Proliferación de los Bacilos Tuberculosos

Los compuestos sulfhidrílicos, y en particular la glutianona, o inhiben absolutamente la proliferación de los bacilos tuberculosos inosiacido-resistentes, catalaso-negativos o no dejan que el desarrollo ocurra más que en discos debajo de un medio sintético semisólido, de acuerdo con la concentración del compuesto sulfhidrílico.

La catalasa y el piruvato antagonizan esos efectos de la glutianona sobre los microbios catalaso-negativos en dicho medio y hacen que que esos microbios se comporten como fenocopias de bacilos tuberculosos catalaso-positivos.

Los efectos antibacterianos de la glutaniona en esas condiciones son atribuibles primariamente a su capacidad para producir peróxido de hidrógeno sobre la oxidación debida al oxígeno molecular.

Suministran estas observaciones una posible explicación bioquímica de la menor patogenicidad de las cepas catalaso-atenuadas así como de las catalaso-negativas en ciertos huéspedes.

RESUME

Les effets de quelques composés sulfhydryle sur la croissance des bacilles tuberculeux

Les composés sulfhydryles en particulier le glutathion, ou bien inhibent complètement la croissance des bacilles tuberculeux isoniazido-résistants et catalase-négatifs ou ne permettent leur croissance qu'en disques placés au-dessous de la surface d'un milieu synthétique semi-solide. La concentration du composé sulfhydryle intervient dans le développement.

La catalase et le pyruvate sont des antagonistes de l'action du glutathion sur les organismes catalases négatifs dans ce milieu et obligent ces germes à se comporter comme des phénocopies de bacilles tuberculeux catalases-positifs.

L'action antibactérienne du glutathion dans ces conditions est attribuée en premier lieu à sa capacité de produire le peroxyde d'hydrogène par oxydation de l'oxygène moléculaire.

Ces observations procurent l'explication biochimique possible d'une réduction de pathogénicité des souches à catalase réduite et des souches catalases-négatives chez certains hôtes.

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STUDIES ON THE RATE OF GROWTH OF MYCOBACTERIA[1]

I. Generation Time of *Mycobacterium Tuberculosis* on Several Solid and Liquid Media and Effects Exerted by Glycerol and Malachite Green

J. M. GUTIÉRREZ-VÁZQUEZ

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INTRODUCTION

In the older studies on mycobacteria the estimation of the amount of growth was essentially subjective and therefore open to many interpretative errors. This lack of quantitation was responsible, in part, for the paucity of information on the nutritional requirements of these microorganisms and the effect of growth-inhibiting substances. The determination of the suitability of various culture media to be used in the routine bacteriology of tuberculosis was even more difficult.

Dubos (1) provided a helpful procedure for the study of some aspects of the metabolism and nutritional requirements of tubercle bacilli by adding Tween® 80 (polyoxyethylene sorbitan monooleate) to the formula of a modified Kirschner medium. Dispersed growth of tubercle bacilli was obtained which made possible the use of photoelectric instruments for the determination of the amount of growth reached at a given time (2, 3). However, this procedure is only applicable to liquid media containing Tween 80, and it is known that this surface-active agent may markedly affect the action on the multiplication of tubercle bacilli of growth-promoting and growth-inhibiting substances (4–8).

More recently, Youmans and Youmans (9) reported a method for the determination of the rate of growth of *M. tuberculosis* in liquid media, which was based upon the determination of the time necessary for several small inocula of tubercle bacilli to grow until they comprised a certain standard mass. This method was potentially applicable also to solid media and did not require the addition of any special substance. Its simplicity and reproducibility made it a valuable tool in the study of those problems still in a state of controversy because of lack of precise data. The method was slightly modified and then successfully applied to the growth of tubercle bacilli on solid media by Gutiérrez-Vázquez and Bojalil (10).

The purpose of this paper is to report some of the results obtained by the use of the small inocula method described by Youmans and Youmans for the determination of the growth rate of *M. tuberculosis*. The observations were made during the experimental study of several solid culture media (Löwenstein-Jensen-Holm, Löwenstein, Petragnani-McNabb, and Herrold), several synthetic liquid media (Sauton, Proskauer-Beck, and Long-Seibert), of a growth-promoting substance (glycerol), and of a growth-inhibiting substance (malachite green).

[1] From the Laboratorio de Quimioterapia Experimental, Escuela Nacional de Ciéncias Biológicas, Instituto Politécnico Nacional, México, D. F., México.

MATERIALS AND METHODS

Generation Time of M. Tuberculosis on Several Solid Egg Media

Media: Several of the media most commonly employed in the routine bacteriology of tuberculosis were studied, such as the Herrold egg yolk-agar medium (11), the Petragnani-McNabb medium (12), the original medium of Löwenstein (13), and the same medium as modified by Jensen and Holm (14, 15). Because of the technical difficulties which arise in attempting to obtain a homogeneous distribution of the inoculum upon the surface of slanted solid media, the media were solidified in 20 by 100 mm. Petri dishes containing 50 ml. portions of the medium under study. Such large volumes aid in counteracting the deleterious effects of desiccation resulting from prolonged incubation of cultures. Portions of the egg yolk-agar medium were poured into the Petri dishes at 45°C. and allowed to solidify. The other three media were inspissated at 85°C. for twenty minutes. The original procedures for the inspissation and sterilization of these media were not followed since variations in the temperature of coagulation and in the number and length of periods of heating produced variations in the chemical composition of the egg media as well as in their ability to promote the growth of tubercle bacilli (16). The plates were stored for twenty-four hours at 37.5°C. to test their sterility and were then used.

Pure culture studies: The H37Rv strain of *M. tuberculosis* was used. Suspensions of 14- to 21-day-old cultures of this strain were prepared and standardized in a Hopkins vaccine tube as described by Youmans and Karlson (17). From this suspension, dilutions were made with distilled water so that the inocula to be employed (0.05 ml.) contained, respectively, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} mg. tubercle bacilli wet weight. Drops 0.05 ml. in size were seeded four to the plate (one drop per quarter disc), using two plates for each quantity of bacilli, i.e., eight seedings for each inoculum on each culture medium. The moisture of the inoculum was allowed to evaporate and the Petri dishes were inserted in polyethylene bags which were then sealed and incubated at 37.5°C. until the appearance of growth was detected from each inoculum on each medium. The first manifestation of growth was the appearance upon the site of inoculation of a matted circular zone, very pale yellow in color. This can be easily observed if the media are examined daily under an illumination which falls obliquely upon the surface of the plates. The time of the first appearance of such growth for each inoculum on each medium was recorded and then plotted against the logarithm of the inoculum. The slope of the straight line so obtained for each medium represents the growth-rate constant of the tubercle bacilli on that medium. From this constant (K), the generation time (G) was calculated as proposed by Youmans and Youmans (9).

It appears that the use of Petri dishes is most convenient for this type of study inasmuch as readings can be more easily made than upon the surface of slanted cultures. The difficulties encountered in obtaining an homogeneous distribution of the inoculum in a slanted surface have already been mentioned. In the case of plate media contained in Petri dishes, it is sufficient to allow a drop containing the inoculum to fall upon the plate in order for it to occupy a more or less reproducible circular zone upon the media being studied.

It is important to point out that the method is based upon the fact that inocula of decreasing size require proportionally greater incubation times to reach a given amount of mass, that is, to reach the minimum growth observable by the naked eye. When very dilute inocula are employed, i.e., 10^{-7} or 10^{-8} mg. wet weight, upon solid media, the appearance of growth does not take place in the form of a matted circular zone, but as a group of isolated colonies. In other words, the time of the appearance of growth from these diluted inocula does not represent the time necessary to reach the same quantity of mass achieved by the larger inocula, although the points corresponding to the dilute inocula do fall upon the growth rate curve obtained from the data furnished by the more concentrated inocula (10^{-1} to 10^{-6} mg. wet weight). Nevertheless, the data obtained with the use of very dilute inocula are of considerable interest because many pathologic specimens contain very few tubercle bacilli.

Isolation studies: In these experiments Herrold medium was not used due to the irregular results obtained with the H37Rv strain. Only the Petragnani-McNabb, the Löwenstein, and the Löwenstein-Jensen-Holm media were studied. Sputum specimens with a high bacillary content from untreated tuberculous patients were selected. The specimens were concentrated and decontaminated by the sodium hydroxide method. That the acid-fast bacilli observed in the sputums were tubercle bacilli was demonstrated by the characteristics of the colonies which later developed on solid media and by virulence tested upon apparently healthy tuberculin-negative guinea pigs.

The inocula used were 0.05 ml. drops of tenfold dilutions of the sediment from the concentrate, which was neutralized and resuspended in distilled water. The actual numbers of tubercle bacilli present in such dilutions were not determined and the logarithms of the dilutions used were employed in the calculations instead of the unknown logarithms of the inocula. This method in no way alters the results. The procedure followed in the remainder of this experiment is identical to that used with the pure strain.

The Effects of Glycerol on the Generation Time of M. Tuberculosis Growing in Sauton Medium

Generation time in several synthetic liquid media: In choosing a medium suitable for the adequate study of the effects of glycerol, comparative determinations of the generation time of H37Rv strain of *M. tuberculosis* were carried out upon several synthetic liquid media containing glycerol as the sole source of carbon. The determinations included the Proskauer-Beck medium (18) as modified by Youmans (19), as well as the Sauton (20), and the Long-Seibert (21) media. The inocula were prepared in the same manner as described for the pure culture studies on solid egg media. The time of first appearance of the typical subsurface granular growth for each inoculum in each medium was recorded and then plotted against the logarithm of the inoculum. From this, the growth rate constant and the generation time of the bacilli for each medium were calculated as described by Youmans and Youmans (9).

The effects of various concentrations of glycerol: The generation time of the H37Rv strain of tubercle bacilli was determined in six different lots of Sauton medium which were identical except in the quantities of glycerol that they contained. The concentrations of glycerol studied were 0.0, 2.0, 4.0, 4.8, 6.0, and 8.0 per cent v/v. Four and eight-tenths per cent v/v glycerol (6.0 per cent w/v) is the concentration recommended for the Sauton medium.

The Effect of Malachite Green upon the Generation Time of M. Tuberculosis Growing on the Löwenstein-Jensen-Holm Medium

Since the Löwenstein-Jensen-Holm medium is one of the most widely used in the bacteriologic diagnosis of tuberculosis and since malachite green is the agent inhibitory to the secondary flora of pathologic products most commonly employed, the effect of this bacteriostatic agent upon the generation time of tubercle bacilli on this medium was studied. The same procedure was followed as utilized in the pure culture studies of the generation time of *M. tuberculosis* in several solid egg media described above. The rate of growth of the H37Rv strain was determined on the Löwenstein-Jensen-Holm medium with malachite green at dilutions of 1:1,000; 1:2,000; 1:4,000; 1:6,000; and without dye.

RESULTS

In table 1 are shown the results of four pure culture experiments on the generation time of *M. tuberculosis* growing on several solid egg media. The Löwenstein-Jensen-Holm medium was the most effective in promoting the growth of tubercle bacilli; the original medium of Löwenstein and the Petragnani-McNabb medium were less effective. Data on the Herrold medium do not appear in table 1 due to the irregularity of the results, which consisted in the appearance of small isolated colonies at different times. The appearance of the matt circular zone took place very belatedly in the case of the low dilutions (inocula of 10^{-1}

TABLE 1

GROWTH-RATE CONSTANTS (K) AND GENERATION TIMES (G)* OF THE H37Rv STRAIN OF M. TUBERCULOSIS ON LÖWENSTEIN-JENSEN-HOLM (LJH), LÖWENSTEIN (L), AND PETRAGNANI-McNABB (PMcN) MEDIA

Media	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
	K	G	K	G	K	G	K	G
LJH	0.400	18.06	0.385	18.74	0.400	18.06	0.400	18.06
L	0.353	20.47	0.375	19.27	0.353	20.47	0.364	19.85
PMcN	0.300	24.07	0.286	25.20	0.300	24.07	0.300	24.07

* Expressed in hours

to 10^{-5} mg. wet weight) and not at all in the high dilutions (inocula of 10^{-6} to 10^{-8} mg. wet weight). All of this prevented the determinations of the time of appearance of growth, a determination without which the rate of growth of tubercle bacilli could not be calculated for this medium.

The data obtained in five isolation experiments on the generation time of *M. tuberculosis* growing on several solid egg media are shown in table 2. There were some differences in the generation times from one strain to the other, but the results were essentially similar to those obtained in the pure culture studies.

In table 3 may be seen the results obtained in a duplicate experiment on the

TABLE 2

GROWTH-RATE CONSTANTS (K) AND GENERATION TIMES (G)* OF 5 STRAINS OF M. TUBERCULOSIS GROWING ON LÖWENSTEIN-JENSEN-HOLM (LJH), LÖWENSTEIN (L), AND PETRAGNANI-McNABB (PMcN) MEDIA, AT THE TIME OF ISOLATION FROM SPUTUM SPECIMENS

Media	Strain 1		Strain 2		Strain 3		Strain 4		Strain 5	
	K	G	K	G	K	G	K	G	K	G
LJH	0.333	22.34	0.286	25.20	0.364	19.92	0.308	23.52	0.235	30.72
L	0.308	24.14	0.250	28.80	0.333	22.34	0.286	25.20	0.200	36.00
PMcN	0.182	40.87	0.154	46.80	0.200	36.00	0.166	43.44	0.143	50.40

* Expressed in hours

TABLE 3

GROWTH-RATE CONSTANTS (K) AND GENERATION TIMES (G)* OF THE H37Rv STRAIN OF M. TUBERCULOSIS IN SAUTON (S), PROSKAUER-BECK (PB), AND LONG-SEIBERT (LS) SYNTHETIC LIQUID MEDIA

Media	Experiment 1		Experiment 2	
	K	G	K	G
S	0.385	18.77	0.375	19.20
PB	0.385	18.77	0.375	19.20
LS	0.312	23.16	0.300	24.07

* Expressed in hours

TABLE 4

GROWTH-RATE CONSTANTS (K) AND GENERATION TIMES (G)* OF THE H37Rv STRAIN OF M. TUBERCULOSIS IN SAUTON SYNTHETIC LIQUID MEDIUM CONTAINING SEVERAL CONCENTRATIONS OF GLYCEROL

Concentrations of Glycerol (v/v)	Experiment 1		Experiment 2	
	K	G	K	G
per cent				
0.0	—	—	—	—
2.0	0.400	18.06	0.385	18.77
4.0	0.400	18.06	0.385	18.77
4.8	0.385	18.77	0.375	19.20
6.0	0.333	22.34	0.307	23.52
8.0	0.286	25.20	0.266	27.12

* Expressed in hours

generation time of tubercle bacilli in several synthetic liquid media. The H37Rv strain grew at the same rate in the Sauton and in the Proskauer-Beck media. Growth was somewhat slower in the Long-Seibert medium. The Sauton medium was chosen for the study of the effects of glycerol.

The results of two experiments on the effects of various concentrations of glycerol are shown in table 4. The H37Rv strain of tubercle bacilli grew at the same rate when the glycerol concentration was 2.0 or 4.0 per cent v/v in the medium employed. A concentration of 4.8 per cent v/v (6.0 per cent w/v) began to retard growth, while at 6.0 and 8.0 per cent v/v the inhibitory effect was enhanced.

The results obtained in one experiment on the effects of malachite green are shown in table 5. They reveal that the H37Rv strain of tubercle bacilli grew more rapidly in a Löwenstein-Jensen-Holm medium devoid of malachite green, and that its generation time increased with increasing concentrations of dye. In spite of this effect, even the lowest inocula in bacilli (10^{-7} mg. wet weight) did not fail to show growth in the presence of the highest concentration of malachite green (1:1,000).

TABLE 5

GROWTH-RATE CONSTANTS (K) AND GENERATION TIMES (G)* OF THE H37Rv STRAIN OF M. TUBERCULOSIS ON LÖWENSTEIN-JENSEN-HOLM MEDIUM CONTAINING SEVERAL CONCENTRATIONS OF MALACHITE GREEN

Concentrations of Malachite Green	Experiment 1	
	K	G
1:1,000	0.250	28.90
1:2,000	0.300	24.07
1:4,000	0.400	18.06
1:6,000	0.428	16.87
None	0.444	16.27

* Expressed in hours

DISCUSSION

The method employed in these experiments has been fully discussed elsewhere (9, 22, 23). Only a few comments will be made here regarding the determination of the rate of growth of the tubercle bacillus on solid media and the results obtained.

The method described is so simple, yields such essentially reproducible results, and is so adaptable for use upon such a variety of types of culture media, that the question is worthy of consideration whether the determination of the rate of growth should not be more commonly used in the elucidation of various aspects of our knowledge of mycobacteria. Thus, such phrases as "rapidly growing acid-fast bacilli" or "a very slow-growing strain of tubercle bacillus" are commonly encountered in the literature. The substitution for such vague and confusing terms of a quantitative expression such as the generation time upon a standard type of medium would give a much clearer and more precise idea of what the writer is trying to convey.

The method proposed in this paper for the determination of the rate of growth of the tubercle bacilli upon solid media at the time of isolation offers the possibility of studying the supposed adaptation of the organism to artificial culture media without requiring previous subculturing upon other media. Such an adaptation implies an increased growth rate of the organism upon such media. In the Youmans and Youmans studies (24), previous subculturing on other media was used.

It seems that the Herrold medium is of little adequacy for the development of the tubercle bacilli from small inocula. The results obtained with the Löwenstein-Jensen-Holm, Löwenstein, and Petragnani-McNabb media confirm those of the most recent investigations carried out under routine conditions (12, 25). Moreover, the results likewise are in agreement with the observations of Cummings, Drummond, and Lewis (16), who found the Löwenstein-Jensen-Holm medium superior to that of Petragnani-McNabb for the growth of the tubercle bacilli, a fact correlated with the higher content in ether-soluble fatty acids of the Petragnani-McNabb medium after inspissation.

The experiments on the effect of glycerol on the growth rate of the tubercle bacilli suggest a concentration of 2.0 to 4.0 per cent v/v as optimum for the development of this organism on the Sauton medium. Older studies reported a progressive improvement of growth on this medium when the concentration of glycerol rose from 0.3 to 5–6 per cent, the latter range being the optimum (26, 27). However, such investigations required the use of prolonged periods of incubation and involved the weighing of the superficial pellicle developed by the bacilli as the criterion of growth. The results put forth in the present paper confirm those of Youmans and Youmans (28), who also found the same growth rate for concentrations of glycerol from 0.4 to 4.0 per cent w/v. Higher concentrations were not employed by these writers.

It appears that malachite green retards the growth of tubercle bacilli, but that its tuberculocidal action is virtually nil, inasmuch as the lowest inocula (10^{-7} mg. wet weight) developed in the presence of a high concentration of the dye

(1:1,000). It is possible that the microorganism possesses a low permeability to the dye, a mechanism which would prevent toxic accumulations in the zones of metabolic activity. Thus, the concentrations of malachite green present in the interior of the organism would be below the bacteriostatic threshold, but high enough to retard the growth of the bacilli.

SUMMARY

A series of studies has been carried out on the growth of tubercle bacilli, using the small-inocula method for the quantitative determination of the rate of growth of the organisms.

The generation time (G) of the H37Rv strain of *M. tuberculosis* was determined on several solid egg media. It was found that this strain grows more rapidly on the Löwenstein-Jensen-Holm medium (G = 18.06 hours), and more slowly on the Löwenstein (G = 19.27–20.47 hours) and on the Petragnani-McNabb (G = 24.07 hours) media. The organism grew poorly and very irregularly on Herrold egg yolk-agar medium; the growth of tubercle bacilli on this medium is more or less consistent only when initiated by large inocula, a fact which made it impossible to determine the generation time of the strain on this medium.

Similar determinations were carried out using as inocula tenfold dilutions of the resuspended sediment of heavily positive sputum concentrates. The generation times on the same media were somewhat higher, but the classification of the media with respect to their growth-promoting capacity toward the tubercle bacilli were identical.

Malachite green possessed a retarding effect upon the growth rate of the H37Rv strain of tubercle bacilli, especially at high concentrations (1:1,000 and 1:2,000). However, its tuberculocidal action was almost nil, for even the smallest inocula (10^{-7} mg. wet weight) were able to develop in the presence of high concentrations of the dye (1:1,000).

SUMARIO

Estudios de la Velocidad del Desarrollo de las Micobacterias: I. Tiempo de Generación del Mycobacterium Tuberculosis en Varios Medios Sólidos y Líquidos y Efectos Ejercidos por el Glicerol y el Verde de Malaquita

Esta serie de estudios versa sobre el desarrollo de los bacilos tuberculosos, usando el método de inóculos pequeños para la determinación cuantitativa de la velocidad del crecimiento de los microbios.

El tiempo de generación (G) de la cepa H37Rv del *M. tuberculosis* fué determinado en varios medios sólidos de huevo. Observóse que esta cepa crece más rápidamente en el medio de Löwenstein-Jensen-Holm (G = 18.06 horas) y más lentamente en los medios de Löwenstein (G = 19.27–20.47 horas) y de Petragnani-McNabb (G = 24.07 horas). El microorganismo creció mal y muy irregularmente en el medio de yema de huevo-agar de Herrold; la proliferación de los bacilos tuberculosos en éste no fué más o menos constante sino cuando se iniciaba con inóculos grandes, hecho este que impidió determinar el tiempo de generación de la cepa en este medio.

Ejecutáronse determinaciones semejantes usando como inóculos diluciones al décimo del sedimento resuspendido de concentrados de esputo intensamente positivo. Los tiempos de generación en los mismos medios resultaron algo más altos, pero fué idéntica la clasificación

de los medios con respecto a su capacidad para fomentar el desarrollo de los bacilos tuberculosos.

El verde de malaquita mostró efecto retardador sobre la velocidad del desarrollo de la cepa H37Rv del bacilo tuberculoso, sobre todo a concentraciones altas (1:1,000 y 1:2,000). Sin embargo, su acción tuberculocida fué casi nula, pues hasta los inóculos más pequeños (10^{-7} mg. peso neto) fueron capaces de proliferar cuando había concentraciones elevadas del colorante (1:1,000).

RESUME

Études sur la vitesse de développement des mycobactéries: I. Temps de reproduction du Mycobacterium tuberculosis sur divers milieux solides et liquides et les effets exercés par le glycérol et le vert d'aniline

Il a été effectué une série d'études sur le développement des bacilles tuberculeux en utilisant la méthode du petit inoculum pour la détermination quantitative de la vitesse de développement de l'organisme.

Le temps de reproduction "generation time" (G) de la souche H37Rv de *M. tuberculosis* fut déterminé sur différents milieux solides à l'oeuf. L'observation montre que cette souche pousse plus vite sur le milieu de Löwenstein-Jensen-Holm (G = 18,06 heure) et plus lentement sur les milieux de Löwenstein (G = 19,27–20.47 heure) et de Petragnani-McNabb (G = 24,07 heure). Le développement du bacille était médiocre et très irrégulier sur le milieu au jaune d'oeuf-gélose de Herrold; sur ce milieu, une certaine uniformité dans le développement du bacille tuberculeux n'est observée que lorsque l'inoculum est volumieux, ce qui a prévenu la détermination du temps de reproduction de cette souche sur ce milieu.

Des déterminations analogues ont été pratiquées en utilisant comme inoculum des dilutions au dixième du sédiment de crachats positifs homogénéisés remis en suspension. Les temps de reproduction sur les mêmes milieux furent un peu plus élevés, mais la classification des milieux selon leur aptitude à favoriser le développement de bacilles tuberculeux était identique.

Le vert d'aniline exerçait une action "retard" sur la vitesse de développement de la souche H37Rv, surtout à concentrations élevées (1 pour 1,000 et 1 pour 2,000). Toutefois, son action tuberculicide était presque nulle car le plus petit inoculum (10^{-7} mg. poids humide) pouvait se développer en présence du colorant à haute concentration (1 pour 1,000).

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THE ANTITUBERCULOUS ACTIVITY OF THIOETHYL COMPOUNDS[1]

MORRIS SOLOTOROVSKY, SEYMOUR WINSTEN, ELLIOTT J. IRONSON, AND HORACE D. BROWN

With the technical assistance of Thomas H. Wombolt and Clinton H. Eary

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INTRODUCTION

The investigation of thioethyl compounds for antituberculous activity emerged from a study of the inhibiting action of alkylmercapto acids on nitrifying bacteria (1). One of these, β-ethyl mercapto propionic acid, was tested for antituberculous activity in mice and was found effective. A large number of thioethyl compounds were synthesized and studied for antituberculous activity in mice. A brief report of the scope of activity of thioethyl compounds, studied by investigators in this laboratory, was presented by Brown and associates (2) and was followed by a more comprehensive description of antituberculous activity of S-ethyl-L-cysteine (3).

Two investigators, however, were unable to confirm the activity of S-ethyl-L-cysteine. A study of this problem revealed that the activity of S-ethyl-L-cysteine and other members of the series was dependent upon formation of a volatile compound. The accumulation of effective concentrations of this substance was achieved apparently by the methods of the present investigators which differed from those used by the other investigators who tested S-ethyl-L-cysteine. A brief report on the correlation of antituberculous activity of the thioethyl compounds with their metabolic cleavage to the volatile compound ethyl mercaptan has been presented by Oginsky and co-workers (5). The studies of del Pianto on sodium ethyl thiosulfate have previously been noted (6).

Subsequent to earlier reports from this laboratory and the reports of del Pianto, Kushner and associates (7) confirmed the antituberculous activity of thioethyl compounds. The present report will give data on the activity of the various thioethyl derivatives studied in this laboratory as conditioned by a slowly ventilating atmosphere. Chemical aspects of the investigation are discussed in separate reports (8).

MATERIALS AND METHODS

In vivo tests: Mycobacterium tuberculosis, human strain H37Rv, was used as the infecting organism for *in vivo* tests. The virulence of the culture has been maintained by serial passage of pellicle growth in Proskauer Beck medium. The culture for inoculation of test animals was prepared by growing pellicle in Dubos Tween®-albumin medium. After ten to fourteen days of incubation at 37° C., the cultures were adjusted with a Coleman 6A spectrophotometer to permit 70 to 80 per cent transmission of light at 620 mμ.

Barckmann IS 32 white mice, weighing 15 to 17 gm., were inoculated intravenously with 0.25 ml. amounts of standardized cell suspension. Test mice in groups of 4 were main-

[1] From the Merck Institute for Therapeutic Research, and the Research Laboratories, Chemical Division, Merck & Co., Inc., Rahway, New Jersey.

tained in glass jars, 6 inches square by 8 inches high, with an opening of 4.5 inches in diameter. The jars were closed with metal screw-cap lids pierced by a central aperture, 0.75 inch in diameter, and two lateral apertures, each 1.25 inches in diameter. The two larger apertures accommodated stainless steel wire mesh vent wells, 1.75 inches long, which were loosely plugged with nonabsorbent cotton. The third opening was closed by a rubber stopper pierced to accommodate the stem of an inverted water bottle. The animals were bedded on bagasse.

For testing oral efficacy, compounds were incorporated into a standard mouse diet mixture[2] and fed *ad libitum*. To determine efficacy by the subcutaneous route, compounds were dissolved or suspended in distilled water at concentrations yielding the desired daily dose in 0.25 ml. of solution and were administered once daily five days per week beginning on the day after infection.

The tests were continued until 50 per cent of the infected controls were dead. Efficacy of chemotherapy was evaluated on the basis of gross pathologic lung involvement. All of the animals which died during the course of the experiment as well as those which survived the test period were submitted to autopsy, and the lungs were observed for gross pathologic involvement with a 2× lens after fixation in 10 per cent formalin for forty-eight hours. The lung lesions were rated as follows:

0 = no involvement
1 = minimal involvement, 10 per cent of tissue tuberculous
2 = moderate involvement, 10 to 25 per cent of tissue tuberculous
3 = moderate to advanced involvement, 25 to 50 per cent of tissue tuberculous
4 = advanced involvement, more than 50 per cent of tissue tuberculous

In vitro tests were performed with both Dubos Tween-albumin medium and Youmans modification of Proskauer-Beck medium. Solutions of water-soluble compounds were sterilized by passage through a Seitz EK filter, and water-insoluble compounds were dissolved or suspended in organic solvents. Series of dilutions were prepared in 10 ml. amounts of medium and the tubes were then inoculated with 0.1 ml. amounts of 1:10 dilutions of culture in Dubos Tween-albumin medium adjusted to permit 90 per cent transmission of light at 620 mμ. The test series was observed for the presence of growth after incubation for fourteen days at 37°C.

RESULTS

In the first trial with β-ethyl mercapto propionic acid, the compound was administered in diet at concentrations of 0.1 and 0.5 per cent. A gross lung lesion score of 0.6 was obtained at the 0.1 per cent level and a score of 0.0 was obtained at the 0.5 per cent level. A more detailed trial demonstrated that this compound was more active than pyrazinamide and para-aminosalicylic acid

[2]

S₁ Diet	*Per Cent*
Yellow corn meal	31.0
Whole wheat flour	30.0
Casein, technical	10.0
Soybean meal	10.0
Linseed oil meal	7.0
Yeast, dried brewers	5.0
Alfalfa meal	2.0
NaCl	0.5
$CaCO_3$	0.5
Mazola oil	3.0
Cod liver oil	1.0
10 mg. vitamin B_2 per kg.	

TABLE 1

COMPARISON OF ANTITUBERCULOUS ACTIVITY OF β-ETHYL MERCAPTO PROPIONIC ACID WITH PAS, PYRAZINAMIDE, AND ISONIAZID

Compound	Gross Lung Score						
	Per Cent Concentration in Diet						
	0.5	0.1	0.05	0.01	0.005	0.001	0.000
β-ethyl mercapto propionic acid	—	0.8	3.2	4.0	4.0	—	—
PAS	3.1	4.0	—	—	—	—	—
Pyrazinamide	0.0	3.2	—	—	—	—	—
Isoniazid	—	—	—	0.0	0.7	—	—
Infected control	—	—	—	—	—	—	4.0
Normal control	—	—	—	—	—	—	0.0

(PAS) and less than isoniazid (table 1). This trial required forty-six days for completion. The average score at 0.1 per cent concentration was 2.0 ± 1 as compared with an untreated control score of 4.0 in infections characterized by an ST_{50} of twenty to twenty-five days.

For further analysis of the chemical groups associated with activity, β-ethyl mercapto propionic acid was divided into components as follows:

C—C	—S—	C—C—	C
1	2	2	4

Approximately 100 compounds were tested in this phase of the investigation. It was found that changes in the first component, involving alkyl substitutions from methyl to octadecyl, or aryl, or heterocyclic substitutions, resulted in a complete loss of activity. Changes at two, involving substitution of nitrogen or oxygen for *S* or higher oxidative states of *S*, also led to loss of activity. However, as will be seen later, the oxidative state of *S* in hydantoin derivatives could be raised with retention of activity. Some changes in the third or fourth fragments could be effected with retention of activity but did not lead to improved performance.

In the case of S-ethyl-L-cysteine, activity was not increased over β-ethyl mercapto propionic acid, but toxicity was reduced to a level permitting consideration of the compound for trial in man. Increasing the carbon chain length of component 3 led to loss of activity. The permissible changes at position 4 included alterations to salts, esters, hydrazides, aldehydes, and alcohols.

The results obtained with some representative compounds of the types noted are summarized in table 2. No significant activity was observed with any of these compounds at a level of 0.05 per cent. The hydrazide, aldehyde, and α-amino derivatives of β-ethyl mercapto propionic acid were comparable in efficacy, showing good activity at 0.1 per cent concentration in diet. The ester was not active at 0.1 per cent concentration, but showed high activity at 0.2 per cent concentration. The salts and the alcohol were less active than other

TABLE 2

ACTIVITY OF DERIVATIVES OF β ETHYL MERCAPTO PROPIONIC ACID

Group	Compound	Gross Lung Score			
		Per Cent Concentration in Diet			
		0.5	0.2	0.1	0.05
Alkyl mercapto acid	β ethyl mercapto propionic acid	0.0		2.6	4.0
Salts	Sodium β ethyl mercapto propionic acid	2.0	4.0	4.0	
	Ca β ethyl mercapto propionic acid		3.8	4.0	
Hydrazides	β ethyl mercapto propionic hydrazide	1.0		1.7	
Esters	Methyl β ethyl mercapto propionate	0.0	0.9	4.0	4.0
Aldehydes	β ethyl mercapto propionic aldehyde		0.3	1.4	3.5
Alcohols	3 ethyl mercapto propanol	0.6	3.2	4.0	
α amino	S ethyl L cysteine	0.0	0.3	2.1	

derivatives as components of diet, requiring 0.5 per cent concentration for demonstration of activity.

In view of the water solubility of the salts, they were tested by the subcutaneous route as well as by the oral route. The activity of the salts by the paren-

TABLE 3

ACTIVITY OF S-ETHYL-L-CYSTEINE DERIVATIVES

Group	Compound	Gross Lung Score		
		Per Cent Concentration in Diet		
		0.2	0.1	0.05
Configuration of α carbon	S ethyl L cysteine	0.5	2.6	3.9
	S ethyl DL cysteine	0.1	2.0	4.0
Ester	Ethyl ester of S ethyl L cysteine	0.9		
Ester hydrochloride	S ethyl L cysteine ethyl ester hydrochloride	1.9	4.0	
N acyl derivative	N formyl S ethyl L cysteine	2.8		
	N acetyl S ethyl L cysteine, sodium salt	3.1	3.9	4.0
	N carboxy S ethyl L cysteine, Ca salt	0.1	3.5	4.0
Hydantoins	S L 5 (ethyl mercapto methyl) hydantoin	0.3	1.3	4.0
	S DL 5 (ethyl mercapto methyl) hydantoin	0.6	1.2	4.0
	S 1 acetyl 2 thio 5 ethyl thio methyl hydantoin	0.5	2.3	4.0
	OS—L 5 ethly sulfinyl methyl hydantoin	1.1		

teral route was approximately one-fifth the activity of dihydrostreptomycin. Both the calcium and sodium salts produced nearly complete suppression of macroscopic pulmonary tuberculous lesions at 5 mg. per mouse per day (scores of 0.3 and 0.4, respectively). At 3 mg. per mouse per day the calcium salt showed

TABLE 4

ACTIVITY OF ETHYL THIOESTERS

Group	Compound	Gross Lung Score				
		Per Cent Concentration in Diet				
		0.5	0.2	0.1	0.05	0.01
Alkyl acids	Ethyl thioacetate		0.6			
	Ethyl thiolaurate		0.0	0.8	4.0	
	Ethyl thiostearate		2.5	1.5	4.0	4.0
	Ethyl mercapto α ethyl butyrate			0.0		
	Ethyl thioester of cyclohexane carboxylic acid		0.0		0.1	4.0
	Hydantoin-5-(acetic acid) ethyl thioester		0.2	2.0	3.9	4.0
	D-camphoric acid ethyl thioester		2.6	4.0		
	Ethyl-3,5,5-trimethyl thiol-hexanoate		0.2	0.3	0.8	4.0
	Ethyl diphenyl thioacetate	1.8	4.0			
	Diethyl di-thiooxalate			0.1	0.0	2.9
Aryl acids	Ethyl thiobenzoate	0.0	0.0	0.0	1.0	4.0
	Ethyl *p*-amino thiobenzoate		0.8	3.3	4.0	
	Ethyl thioanisate		0.2	1.2	3.0	
	Ethyl *p*-amino thiobenzoate		0.1	3.3	4.0	
	Ethyl *p*-(ethyl sulfonyl)-thiobenzoate			0.0	3.9	
	m-nitro ethyl thiobenzoate		0.5			
	NN-di-propyl-*p*-carboxybenzene-sulfonamide ethyl thioester		0.2	3.5	3.6	
Heterocyclic acids	Ethyl thiofuroate		0.0	0.0	0.1	4.0
	5-nitro-2-furoic acid ethyl thioester		0.0	0.2	3.7	
	Thiophene, 2-carboxylic acid ethyl mercaptan ester		0.2			
	Ethyl thionicotinic HCl		0.0	0.0	0.8	4.0
	Ethyl thioisonicotinic HCl		0.0	0.2	3.0	
	Ethyl thiopyrazinoate		0.0	0.0	0.0	4.0
Carbonates	O-S-diethyl thiocarbonate		0.0		0.3	4.0
	O-phenyl-S-ethyl-thiocarbonate		0.2			
	Benzyl ethyl mercapto carbonate		0.2	2.7	4.0	
Xanthates	Ethyl ethyl xanthate		0.0	0.0	1.9	4.0
Immino thio	Ethyl immino thiobenzoate HCl			0.2	2.9	4.0
Thiosulfates	Sodium ethyl thiosulfate *p*-acetyl benzene sulfonyl		0.5			
	Toluene sulfonic acid thioethyl ester		0.0	0.6	3.4	

moderate activity (score 2.1) and the sodium salt gave poor activity (score 3.3). At 1 mg. per mouse per day, the calcium salt gave poor activity (score 3.2), and the sodium salt no activity (score 4.0). Dihydrostreptomycin gave high antituberculous activity at 0.8 mg. per mouse per day (score 0.7), moderate activity at 0.4 mg. (score 1.9), and poor activity at 0.2 mg. (score 2.8).

Since S-ethyl-L-cysteine showed good suppression of gross lung involvement at concentrations of 0.1 to 0.2 per cent in the diet and showed low toxicity in pharmacologic studies a variety of derivatives of this compound was prepared in an attempt to improve antituberculous activity. The results obtained with these compounds are summarized in table 3. It was found that the configuration of the alpha carbon did not alter the level of activity. Activity was retained but not improved by preparing esters or ester hydrochlorides. Stable substitutions on the amino group resulted in loss of activity.

Hydantoins were of special interest because they are intermediates in the synthesis of S-ethyl-L-cysteine. An active hydantoin, 5-n-heptyl-2-thiohydantoin, has been described by Archer and co-workers (9). Among the hydantoins activity was maintained when the oxidative state of *S* was raised to sulfoxide but not to sulfone. Since L-5 ethyl sulfinyl methyl hydantoin is soluble in water, it was tested for activity by the subcutaneous route. It was half as active as dihydrostreptomycin. The sulfinyl compound produced good activity down to 1.25 mg. per mouse per day (score 1.1) but was inactive at 0.6 mg. (score 3.6). Dihydrostreptomycin gave high activity at levels down to 0.6 mg. (score 1.2). This degree of activity by the parenteral route surpassed the activity of S-ethyl-L-cysteine and salts of β-ethyl mercapto propionic acid administered by the same route. Administered in the diet, sulfinyl hydantoin did not appear superior

TABLE 5

ACTIVITY OF MISCELLANEOUS THIOETHYL COMPOUNDS

Group	Compound	Gross Lung Score				
		Per Cent Concentration in Diet				
		0.5	0.2	0.1	0.05	0.01
	2-ethyl mercapto oxazoline		0.6	2.9	4.0	
Hetero components attached to alpha carbon	2-ethyl mercapto benzoxazole		2.3			
	S-ethyl mercapto 4,5-thiazoline		1.1	4.0		
Unsaturated beta carbon	Thioethyl mercapto 1,4 quinone		1.5	2.8	3.6	4.0
Polysulfides	Diethyl disulfide			0.0	0.0	3.7
	Diethyl trisulfide			0.0	0.0	3.3
	Ethyl tert butyl disulfide			1.3	4.0	
	Diethyl tetrasulfide			0.0		
	Ethyl phenyl disulfide			0.3	3.4	
	Ethyl tert amyl disulfide			0.4		
	S-ethyl mercapto cysteine		0.1	2.1	3.5	

TABLE 6

IN VITRO ACTIVITY OF THIOETHYL COMPOUNDS AGAINST M. TUBERCULOSIS HUMAN STRAIN H37Rv

Group	Compound	Inhibiting Concentration
		γ/ml.
Derivatives of β-ethyl mercapto propionic acid	β-ethyl mercapto propionic acid	10
	Methyl-β-ethyl mercapto propionate	25
	β-ethyl mercapto propionic aldehyde	5
	β-ethyl mercapto propanol	>100
Derivative of S-ethyl-L-cysteine	S-ethyl-L-cysteine	>100
	S-ethyl-DL-cysteine	>100
	S-ethyl-L-cysteine ethyl ester HCl	>100
	S-ethyl-*N*-acetyl-L-cysteine, Na salt	>100
	1-acetyl-2-thio-5-ethyl-thiomethyl hydantoin	>100
Thioesters	Ethyl thiobenzoate	>100
	Ethyl thiofuroate	50
	O,S-diethylthiocarbonate	>100
Miscellaneous	S-ethyl mercapto-4,5-thiazoline	50
	Diethyltrisulfide	>100
	Ethyl-t-amyl disulfide	50

to S-ethyl-L-cysteine. Since cleavage of this sulfinyl hydantoin to ethyl mercaptan could not be demonstrated (10), the question whether it might be active by virtue of the thioethyl component cannot be resolved without the use of tubercle bacilli rendered resistant to ethyl mercaptan. Such organisms are not available at present.

The thioesters comprised a further series of thioethyl derivatives which included seven groups of active compounds. The data on these compounds are summarized in table 4. Among alkyl acid esters those prepared from acetic through stearic acid were active. This group included 10 compounds which showed good activity at 0.05 per cent concentration, and were therefore two- to fourfold as active as β-ethyl mercapto propionic acid and S-ethyl-L-cysteine.

Data on miscellaneous thioethyl compounds are given in table 5. The range of active derivatives includes those with hetero components attached to the alpha carbon, with unsaturated beta carbons, and polysulfides. The polysulfides include compounds which are more active than β-ethyl mercapto propionic acid or S-ethyl-L-cysteine.

In vitro activity could not be correlated with *in vivo* activity. The results of *in vitro* tests with representative thioethyl compounds are given in table 6.

SUMMARY

Antituberculous activity in the mouse has been demonstrated for more than 50 compounds possessing a thioethyl component. The first effective compound in this series, β-ethyl mercapto propionic acid, was more active than para-

aminosalicylic acid (PAS) and pyrazinamide but less active than isoniazid. Conversion of β-ethyl mercapto propionic acid to salt, hydrazide, ester, aldehyde, and alcohol yielded active compounds which were not significantly superior to the acid. S-ethyl-L-cysteine was as active as, but less toxic than, β-ethyl mercapto propionic acid. A stereoisomer, an ester, ester hydrochloride, *N*-acyl derivative and hydantoins related to S-ethyl-L-cysteine were also active. Many of the effective thioesters were more active than the first active compound, β-ethyl mercapto propionic acid, with efficacy at 0.05 per cent concentration in diet. This level of activity renders them comparable to streptomycin. A miscellaneous group of active derivatives is also noted.

SUMARIO

La Actividad Antituberculosa de los Compuestos Tioetílicos

Para más de 50 compuestos que poseen un componente tioetílico se ha demostrado que ejercen actividad antituberculosa en el ratón. El primer compuesto eficaz de esta serie, el ácido β-etilo-mercapto-propiónico, resultó más activo que el PAS y la piracinamida, pero menos activo que la isoniacida. La conversión del ácido β-etilo-mercapto-propiónico en sal, hidracida, éster, aldehido y alcohol rindió compuestos activos, pero no mayormente superiores al ácido. La S-etilo-*L*-cisteína fué tan activa como el ácido β-etilo-mercapto-propiónico, pero menos tóxica que éste. Un estéreoisómero, un éster, el clorhidrato del éster, un derivado N-acílico e hidantoínas relacionadas con la S-etilo-*L*-cisteína también resultaron activos. Muchos de los tioésteres eficaces fueron más activos que el primer compuesto activo, el ácido β-etilo-mercapto-propiónico, mostrando eficacia a una concentración de 0.05 por ciento en la alimentación. Este índice de actividad los hace comparables a la estreptomicina. También se menciona un grupo variado de derivados activos.

RESUME

L'activité tuberculostatique des composés thioéthyle

L'activité tuberculostatique de plus de 50 composés possédant un composant thioéthyle a été démontrée chez la souris. Le premier composé efficace de cette série, l'acide β-ethyl mercapto propionique, fut plus actif que le PAS et le pyrazinamide, mais moins actif que l'isoniazide. La conversion de l'acide β-éthyl mercapto propionique en sel, hydrazide ester, aldéhyde et alcool donnait des composés actifs qui n'étaient pas nettement supérieurs à l'acide. La S-éthyl-L-cystéine était aussi active, mais moins toxique, que l'acide β-éthyl mercapto propionique. Un stéréoisomère, un ester chlorhydrique, un dérivé N-acyl et l'hydantoïne apparentée à l'S-éthyl-L-cystéine étaient également actifs. Un grand nombre de ces thioesters efficaces étaient plus actifs que le premier composé actif, l'acide β-éthyl mercapto propionique; la concentration de 0,05 pour cent dans la ration est efficace. Ce degré d'activité les rend comparables à la streptomycine. Un groupe mixte de dérivés actifs est également mentionné.

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EFFECT OF VENTILATION ON ANTITUBERCULOUS ACTIVITY OF S-ETHYL-L-CYSTEINE AND RELATED COMPOUNDS[1]

MORRIS SOLOTOROVSKY

With the technical assistance of Thomas H. Wombolt and Clinton H. Eary

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INTRODUCTION

In 1954 (1) it was reported that a series of thioethyl compounds were active against tuberculosis in mice; S-ethyl-L-cysteine, a member of this series, was selected for further study. Detailed data on this compound were presented in November, 1954 (2). Although S-ethyl-L-cysteine showed less antituberculous activity than other members of the series, it was also less toxic than the more active compounds in the series. The published results were based on the experience in this laboratory of more than 50 repeated trials, and indicated that activity against an experimental infection characterized by a median survival time of approximately twenty days could be regularly expected at 0.2 per cent concentration in diet. Under the test conditions, S-ethyl-L-cysteine was more active than pyrazinamide but less active than isoniazid.

S-ethyl-L-cysteine was subsequently submitted to other investigators, first for trials in guinea pigs (3) and later for trials in mice (4). In the hands of other investigators, S-ethyl-L-cysteine was inactive in guinea pigs and in mice. Experience with pyrazinamide offered a precedent for a lack of correlation between guinea pig and mouse, but the contradictory results with the mouse were puzzling.

The two other investigators and the present writers used the same strain of *M. tuberculosis*, and the experimental infections produced were of similar severity. Two immediately evident points of difference were the basal diet and strain of mice. Comparative trials, however, showed that these differences were not responsible for the divergence of results. Further reflection on other possible differences in experimental conditions led the present investigators to recall that there was a difference in the method of housing mice. Both of the other investigators housed their mice in pans closed above with wire mesh lids, but mice in this laboratory were housed in glass jars which limited the rate of ventilation of air in the jars. The divergence in results was found to depend upon the differences in ventilation in the methods of housing.

MATERIALS AND METHODS

Mycobacterium tuberculosis, human strain H37Rv, was used as the infecting organism for *in vivo* tests. Details regarding culture methods and infection of mice are given in a previous report (5).

Following the usual procedure in this laboratory for testing efficacy by oral route, the

[1] From the Merck Institute for Therapeutic Research, Rahway, New Jersey.

thioethyl compounds were incorporated into a stock diet, designated S_2,[2] or Rockland or Purina commercial mouse ration. One kilogram amounts of test diet were mixed in a rotary mixer and stored in closed gallon jars. For daily administration, 20 gm. of test diet were transferred to 50 ml. glass specimen jars. These jars were open to the atmosphere overnight before transfer to animal jars or pans.

The animals were housed by two methods. In the first method, which is standard in this laboratory, groups of 4 animals each were kept in glass jars, 6 inches square by 8 inches high, with a round opening, 4.5 inches in diameter. The jars were closed by metal screw-cap lids pierced by a central aperture, 0.75 inches in diameter, and two lateral apertures, 1.25 inches in diameter. The two larger apertures accommodated stainless steel mesh wells, 1.75 inches long, which were loosely plugged with nonabsorbent cotton. Thus, air passing from the jar was filtered through the cotton. The third opening was closed by a rubber stopper pierced to accommodate the stem of an inverted water bottle.

In the second method, groups of 8 animals each were housed in stainless pans, 6 inches deep by 10 inches wide by 5 inches high, closed above with wire mesh lids through which a water bottle was inserted. Bagasse was used as bedding for both animal containers.

Groups of 8 mice were used for each dosage level in individual experiments. The procedure for evaluation of efficacy on the basis of inhibition of gross lung involvement is described in reference (5). The lungs were scored as follows:

0 = no involvement
1 = minimal tuberculosis (less than 10 per cent involvement)
2 = moderate tuberculosis (10 to 25 per cent involvement)
3 = moderate to advanced tuberculosis (25 to 50 per cent involvement)
4 = advanced tuberculosis (more than 50 per cent involvement)

RESULTS

To compare the effect of basal diet, S-ethyl-L-cysteine was incorporated into each of the three diets at 0.5 and 0.2 per cent concentration. The mice in these trials were maintained in the standard "closed" glass jar. S-ethyl-L-cysteine was equally effective in all three diets. The lung scores at both levels for each diet were less than 1, thus indicating high efficacy. The infected controls were scored 4.0.

Similarly, efficacy could be demonstrated equally well with different strains of mice, namely Strong and Barckmann IS-32. S-ethyl-L-cysteine was incorporated in Purina commercial mouse ration at 0.5 per cent for presentation to mice of the Strong strain and at the same concentration in the S_2 diet for presentation to Barckmann IS-32 mice. These conditions duplicated both the

[2]

S_2 Diet	Per Cent
Yellow corn meal	31.0
Whole wheat flour	30.0
Casein, technical	10.0
Soybean meal	10.0
Linseed oil meal	7.0
Yeast, dried brewers	5.0
Alfalfa meal	2.0
NeCl	0.5
$CaCO_3$	0.5
Mazola	3.0
Cod liver oil	1.0

10 mg. vitamin B_2 per kg.

TABLE 1

EFFECT OF VENTILATION ON ACTIVITY OF THIOETHYL COMPOUNDS

Compound	Gross Lung Score							
	Open Pan				Closed Jar			
	Per Cent Concentration in Diet				Per Cent Concentration in Diet			
	0.5	0.2	0.1	0.05	0.5	0.2	0.1	0.05
S-ethyl-L-cysteine	4.0	4.0	—	—	0.3	0.8	—	—
Sodium ethyl thiosulfate	—	0.6	1.6	4.0	—	0.4	1.1	4.0
Ethyl thio *p*-ethyl sulfonyl benzoate	—	3.5	—	—	—	2.2	—	—
Ethyl thiofuroate	—	0.6	1.3	3.6	—	0.2	0.0	0.8
Ethyl thiobenzoate	—	0.5	1.5	4.0	—	0.1	0.3	2.4
O S ethyl thiocarbonate	—	2.9	—	—	—	0.0	—	—
Diethyl disulfide	—	0.9	—	3.3	—	0.0	—	0.2

diet and strain factors of one of the cooperating investigators for comparison with the diet and mouse strain in this laboratory. S-ethyl-L-cysteine gave a score of 0.2 with mice of Strong strain and 0.0 with Bareknann IS-32 mice, indicating high efficacy in either strain of mice. The infected controls were scored 4.0.

The effect of housing on the efficacy of S-ethyl-L-cysteine was then compared by maintaining one group of test mice in pans covered with the conventional wire mesh lids and a second group of test mice in glass jars. The summarized results of three trials are shown in table 1. S-ethyl-L-cysteine was highly active at 0.2 per cent concentration in diet when the test mice were maintained in the slowly ventilating atmosphere of the "closed" jar, and no activity was observed at concentrations as high as 0.5 per cent in diet when the mice were kept in pans permitting free ventilation. Activity was essentially eliminated by free ventilation.

This comparative procedure was then extended to other members of the thioethyl series, as shown in table 1. There was wide variation in the influence of ventilation on efficacy. Free ventilation did not reduce the activity of sodium ethyl thiosulfate and had little effect on the activity of ethyl thio *p*-ethyl sulfonyl benzoate. However, free ventilation produced a significant decline in the activity of ethyl thiofuroate, ethyl thiobenzoate, O S ethyl thiocarbonate, and diethyl disulfide. The 0.05 per cent level effective for ethyl thiofuroate, ethyl thiobenzoate, and diethyl disulfide in the "closed" jar was ineffective in the open pan. Similarly, O S ethyl thiocarbonate lost activity at 0.2 per cent concentration in diet when tested in the open pan.

DISCUSSION

The results of this study indicate that S-ethyl-L-cysteine and other thioethyl compounds are active through formation of a volatile compound. S-ethyl-L-cysteine was active only under conditions that favored accumulation of the

volatile compound, whereas other compounds in the series varied in their dependence upon reduced ventilation for demonstration of antituberculous activity. It has already been noted that ethyl mercaptan possesses a high level of activity *in vivo* when given by stomach tube. A subsequent report will show that ethyl mercaptan is highly active when administered as a gas as well as by stomach tube, and supports the hypothesis that S-ethyl-L-cysteine and other thioethyl compounds act through release of ethyl mercaptan.

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THE ANTITUBERCULOUS ACTIVITY OF ETHYL MERCAPTAN[1]

MORRIS SOLOTOROVSKY, ELLIOTT IRONSON, AND SEYMOUR WINSTEN

With the technical assistance of Thomas H. Wombolt and Clinton H. Eary

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INTRODUCTION

In a study of thioethyl compounds for antituberculous activity (1–3), the present investigators postulated that the various derivatives could be metabolized to diethyl disulfide which could be further metabolized to ethyl mercaptan. As a corollary of this hypothesis, it was anticipated that antituberculous activity of the thioethyl compounds would be directly related to their chemical lability to cleavage, with release of ethyl disulfide and ethyl mercaptan. The activity of the disulfides and other polysulfides was readily demonstrated and, in support of the hypothesis, the disulfides and polysulfides were more active than β-ethyl mercapto propionic acid. It was impossible at first to demonstrate activity for ethyl mercaptan. The high volatility of the compound appeared to account for the lack of activity (4, 5). Using appropriate experimental methods, both *in vivo* and *in vitro* activity for ethyl mercaptan has now been demonstrated.

MATERIALS AND METHODS

In vivo tests: Mycobacterium tuberculosis, human strain H37Rv, was used as the infecting organism for *in vivo* tests. Details of culture methods, infection of mice, and method of housing mice are given in a previous report (3).

In tests for antituberculous efficacy as a component of diet, ethyl mercaptan and S-ethyl-L-cysteine were incorporated into standard mouse diet mixture.[2] A 1,000 gram quantity, sufficient for a three-week trial, was prepared at the beginning of each test and stored in screw-capped jars at room temperature. Approximately 20 gm. amounts of diet were placed in 50 ml. specimen bottles each evening for subsequent transfer to mouse jars the next morning.

For tests of antituberculous efficacy by stomach tube administration, dilutions of ethyl mercaptan were prepared in peanut oil to contain the desired daily dose per mouse in 0.25

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[1] From the Merck Institute for Therapeutic Research, Rahway, New Jersey.

[2]

S₇ Diet	*Per Cent*
Yellow corn meal	31.0
Whole wheat flour	30.0
Casein, technical	10.0
Soybean meal	10.0
Linseed oil meal	7.0
Yeast, dried brewers	5.0
Alfalfa meal	2.0
NaCl	0.5
$CaCO_3$	0.5
Mazola oil	3.0
Cod liver oil	1.0

10 mg. Vitamin B_{12} per kg.

ml. of oil solution. The dilutions were prepared daily, not more than one hour before administration. The animals were fed commercial pelleted ration.

For testing antituberculous efficacy by gas inhalation, 10 ml. amounts of appropriate dilutions of ethyl mercaptan in oil were placed in 40 ml. bottles closed with plastic screw caps. The individual caps were pierced by a hole, 0.0625 inch in diameter to permit slow release of ethyl mercaptan gas into the animal jar. The animals were transferred daily to clean jars into which the fresh reservoirs of ethyl mercaptan had been inserted two hours previous to transfer of mice. This procedure permitted the ethyl mercaptan to attain the appropriate equilibrium in the atmosphere of the jar prior to the transfer of mice. Four mice were housed in each jar and two jars of mice were used for each test level in individual trials. These animals were fed commercial pelleted ration.

The procedure for evaluation of efficacy on the basis of inhibition of gross lung involvement is described in reference (3). The lung lesions were scored as follows:

0 = no involvement
1 = minimal involvement, 10 per cent of tissue tuberculous
2 = moderate involvement, 10 to 25 per cent of tissue tuberculous
3 = moderate to advanced involvement, 25 to 50 per cent of tissue tuberculous
4 = advanced involvement, more than 50 per cent of tissue tuberculous

In vitro tests: In vitro testing was performed with Youmans' modification of Proskauer and Beck medium. Propyl mercaptan and butyl mercaptan were added aseptically to the medium and serial dilutions were then prepared as desired. The tubes of culture medium were inoculated with 0.1 ml. amounts of a 1:10 dilution of *M. tuberculosis*, human strain H37Rv, in Dubos Tween®-albumin medium adjusted to permit 90 per cent transmission of light at 620 mμ in a Coleman 6A Spectrophotometer. The incubation of tests with propyl and butyl mercaptan was performed both with closed jars and with free ventilation.

For testing methyl mercaptan and ethyl mercaptan, tubes inoculated with culture prepared as described above were placed in one-gallon screw cap jars. Small beakers containing 10 ml. amounts of dilutions of methyl or ethyl mercaptan in peanut oil were then placed within the jars; these mercaptan solutions were thus not incorporated directly into the medium. The test series was observed for the presence of growth after fourteen days incubation at 37°C.

RESULTS

In the earliest trials, ethyl mercaptan was tested as a component of diet. S-ethyl-L-cysteine given at 0.2 per cent concentration was used as a positive control. In the first trial, ethyl mercaptan was administered at 0.2 per cent concentration. Following the usual procedure in this laboratory, a three-week supply of diet mixture was prepared at the beginning of the experiment. The experimental conditions appeared satisfactory since 4 of 8 infected controls were alive at the end of the nineteen-day test period and the lung score was 4.0. All of the mice treated with S-ethyl-L-cysteine survived with a lung score of 0.0, but ethyl mercaptan was ineffective as an antituberculous drug giving a score of 4.0. In the second trial the concentration of ethyl mercaptan was raised to 0.6 per cent and again ethyl mercaptan was ineffective.

The lack of activity was surprising and it appeared possible that the high volatility of the compound might have been responsible for the negative result. The bulk test diet had been stored in a screw-capped jar at room temperature. Since the daily ration was transferred to small open bottles each evening for subsequent transfer to mouse jars the next morning, however, the actual concentration of ethyl mercaptan in the diet when administered might have been considerably lower than in the original formulation.

TABLE 1

THE ACTIVITY OF ETHYL MERCAPTAN ADMINISTERED BY STOMACH TUBE

Compound	Route of Administration	Gross Lung Score					
		Mg. per Mouse per Day					
		5.0	2.5	1.0	0.5	0.1	0.0
Ethyl mercaptan	Stomach tube	0.0	0.5	0.6	3.1	3.5	
Dihydrostreptomycin	Subcutaneous	—	—	1.1	2.2	2.5	
Infected controls	—	—	—	—	—	—	4.0
Normal controls	—	—	—	—	—	—	0.0

Ethyl mercaptan was then tested for antituberculous activity by stomach tube, using freshly prepared oil solutions. Ethyl mercaptan was initially administered at 10 mg. per mouse per day. Four of the 8 mice in the group died after three days. The daily dose was then lowered to 5 mg. and the 4 remaining mice survived the test period of eighteen days. One of the 4 mice had minimal tuberculosis, the 3 others showed no gross involvement, and the score for the group was 0.2. The infected controls gave a score of 4.0. S-ethyl-L-cysteine at 0.2 per cent concentration in the diet gave a score of 0.1. These results with ethyl mercaptan at 5 mg. per mouse per day were confirmed in a second trial; the gross lung score was 0.1. Further tests were performed to establish the dose response range. The results of these tests using dihydrostreptomycin as the comparative standard are summarized in table 1. Ethyl mercaptan administered by stomach tube compared favorably with dihydrostreptomycin administered by subcutaneous route. The test animals treated with either compound at 1 mg. per mouse per day showed minimal involvement. At doses below 1 mg. the mice treated with ethyl mercaptan showed advanced tuberculous involvement with evidence of only slight drug activity, and those treated with dihydrostreptomycin showed moderate tuberculous involvement.

After it was learned that S-ethyl-L-cysteine was more active when the test mice were maintained in a slowly ventilating atmosphere (4) and that increased ventilation also reduced the activity of other thioethyl compounds, it was postulated that thioethyl compounds might have been converted in the animal into ethyl mercaptan which in turn produced the antituberculous effect. Ethyl mercaptan was therefore tested for activity when administered as a gas.

The results of three trials with ethyl mercaptan gas are summarized in table 2. Dilutions of ethyl mercaptan within the range of 1:10 to 1:5,000 were tested on animals exposed for three, six, and twenty-four hours per day. The concentrations of ethyl mercaptan in the atmosphere of the jars were calculated from direct determination of radioactive S present in the atmosphere following deposition of dilutions of S^{35} ethyl mercaptan in the reservoir jars under comparable conditions.[3] The concentration attained with the 1:10 dilution of ethyl mercaptan was toxic, but lower concentrations were tolerated. With three-hour

[3] We are indebted to Mr. David Woodbury and Dr. Charles Rosenblum of the Research Laboratories, Chemical Division, Merck & Co., Inc., Rahway, New Jersey, for these determinations, performed by an *in situ* method devised for this investigation.

TABLE 2

THE ACTIVITY OF ETHYL MERCAPTAN ADMINISTERED AS A GAS

Dilution of Ethyl Mercaptan	Maximal Concentration Ethyl Mercaptan Parts per Million	Gross Lung Score		
		Hours of Exposure per 24 Hours		
		3	6	24
1:10	2,700	toxic	—	—
1:25	1,500	1.1	1.5	—
1:50	1,000	1.6	—	—
1:100	720	1.9	1.5	0.5
1:500	365	2.4	1.4	0.0
1:1,000	250	2.2	0.9	0.1
1:2,000	200	2.2	0.6	0.0
1:5,000	100	4.0	4.0	3.1

TABLE 3

THE IN VITRO ACTIVITY OF MERCAPTANS

Compound	Method of Presentation	Presence of Growth		
		Ml. in Closed Gallon Jar		
		1	0.1	0.01
Methyl mercaptan	Volatilized in closed atmosphere	—	—	+
Ethyl mercaptan	Volatilized in closed atmosphere	—	—	—
		Concentration γ per Ml.		
		100	50	25
Propyl mercaptan	Incorporated in medium	—	±	+
Butyl mercaptan	Incorporated in medium	+	+	+

exposure periods, good to high activity was observed with dilutions up to 1:100; and moderate activity was observed at 1:2,000 dilution. With six-hour exposure periods, dilutions up to 1:2,000 gave high activity but 1:5,000 dilution was ineffective. When the exposure period was increased to twenty-four hours, nearly complete suppression of gross lung involvement was obtained from dilutions as high as 1:1,000 and even the 1:5,000 dilution was a slightly active concentration.

The results of *in vitro* tests are given in table 3. Methyl and ethyl mercaptan at gas concentrations attained with 10 ml. of 1:100 dilution in one-gallon jars inhibited growth of the tubercle bacilli. This dilution of ethyl mercaptan resulted in atmospheric concentration of 8,050 parts per million. Propyl mercaptan showed partial inhibition at 50 γ per ml. and complete inhibition at 100 γ per ml., but butyl mercaptan was ineffective at 100 γ per ml.

DISCUSSION

The results of this study support the hypothesis that the thioethyl compounds exert their antituberculous activity through conversion into ethyl mercaptan.

Ethyl mercaptan was inactive when incorporated in the diet. It is assumed that ethyl mercaptan evaporated from the diet mixture. To overcome this difficulty it was elected to test activity with administration by stomach tube and it was then possible to demonstrate high activity.

The observation that S-ethyl-L-cysteine is more active in a slowly ventilating atmosphere indicated that antituberculous activity was achieved through formation of a volatile component. Ethyl mercaptan could fit the observed conditions. Accordingly, the activity of ethyl mercaptan was tested when administered as a gas and a high level of antituberculous activity was observed.

Further, ethyl mercaptan, unlike most of the thioethyl compounds, was active *in vitro* when cultures were exposed to the compound in gaseous form. Activity was inversely proportional to the number of carbon atoms in the mercaptan molecule. Ethyl and methyl mercaptan appeared to be the most active and butyl mercaptan the least active member of the limited test series.

This sequence of observations has led to a study to determine whether the thioethyl compounds that are active *in vivo* can be metabolically cleaved to yield ethyl mercaptan by fresh mouse tissue (6). Reasonably good correlation was obtained between therapeutic activity and rapidity of cleavage to ethyl mercaptan. Paradoxically, S-ethyl-L-cysteine was resistant to cleavage, and a second cleavage mechanism involving intervention of intestinal bacteria was studied. Organisms found in the mouse intestine cleaved S-ethyl-L-cysteine to yield ethyl mercaptan.

SUMMARY

Ethyl mercaptan showed high antituberculous activity in mice when administered by stomach tube or as a gas. By stomach tube it was approximately half as active as dihydrostreptomycin administered by parenteral route. When administered as a gas, a concentration of 200 parts per million of ethyl mercaptan was moderately active when mice were exposed to the gas for three hours per day and highly active when mice were exposed continuously to this concentration of gas. Ethyl mercaptan and methyl mercaptan inhibited *M. tuberculosis*, human strain H37Rv, *in vitro*.

SUMARIO

La Actividad Antituberculosa del Mercaptán Etílico

Administrado con sonda gástrica o en forma de gas, el mercaptán etílico mostró elevada actividad antituberculosa en los ratones. Con la sonda gástrica, alcanzó aproximadamente la mitad de la actividad de la dihidroestreptomicina administrada por vía parentérica. Administrado en forma de gas, una concentración de 200 partes por millón de mercaptán de etilo fué moderadamente activa al exponer a los ratones al gas durante tres horas al día y sumamente activa cuando se exponía continuamente a los ratones a esa concentración de gas. El mercaptán etílico y el mercaptán metílico inhibieron *in vitro* el *M. tuberculosis* de procedencia humana, cepa H37Rv.

RESUME

L'activité antituberculeuse de l'éthyl mercaptan

L'éthyl mercaptan présentait un haut degré d'activité chez la souris quand on l'administrait à l'aide d'un tube gastrique ou par inhalation à l'état gazeux. Le degré d'activité

de l'administration par tube gastrique était environ la moitié de celui de la dihydrostreptomycine parentérale. Administré à l'état de gaz, l'éthyl mercaptan à la concentration de 200 pour 1 million avait une activité modérée quand les souris étaient exposées à ce gaz pendant trois heures par jour et une activité élevée quand les souris étaient exposées à cette concentration du gaz d'une façon continue.

L'éthyl mercaptan et le méthyl mercaptan inhibaient *in vitro* le *M. tuberculosis* humain, souche H37Rv.

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METABOLIC CLEAVAGE OF ANTITUBERCULOUS THIOETHYL COMPOUNDS[1]

EVELYN L. OGINSKY, MORRIS SOLOTOROVSKY, AND HORACE D. BROWN

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INTRODUCTION

The antituberculous activity in mice of a wide variety of compounds with the thioethyl group as a common structural component (1) suggested that the antibacterial activity might reside in ethyl mercaptan released within the mouse by metabolic cleavage of these compounds. This hypothesis was supported by the following observations: (*a*) The *in vitro* activity of the thioethyl compounds could not be directly correlated with their *in vivo* activity (1); (*b*) ethyl mercaptan itself was active *in vitro* (2); (*c*) the activity of a representative compound, S-ethyl cysteine, was dependent on restricted ventilation of the mouse housing, in accordance with the high volatility of ethyl mercaptan (3).

Studies were therefore conducted to determine whether those thioethyl compounds active only *in vivo* were metabolically cleaved to yield ethyl mercaptan.

MATERIALS AND METHODS

For studies with mouse tissue, Carworth white mice, weighing approximately 25 gm., were sacrificed by severing the cervical vertebrae. The liver and lungs were immediately removed to cold isotonic potassium chloride solution, blotted dry, and aliquots of approximately 130 mg. of liver, or the entire lungs weighing approximately 110 mg., were minced with a razor blade. The tissue was then placed in the Warburg flask for assay of ethyl mercaptan formation as described below. The time of preparation of tissue, from sacrifice of the mice to insertion in the Warburg flask, was less than ten minutes.

In experiments with bacterial cultures, two laboratory stock strains, *Escherichia coli* strain Murray and *Streptococcus faecalis* strain F24, and two other strains isolated from mouse gut, identified as *E. coli* strain 5 and *S. faecalis* strain 3, were employed. The organisms were grown for eighteen hours at 37°C. in a liquid medium containing 1 per cent Difco® yeast extract, 1 per cent Difco tryptone, 0.5 per cent K_2HPO_4, and 0.1 per cent dextrose, centrifuged, washed once with distilled water, and resuspended in distilled water at a concentration of 1 mg. of bacterial nitrogen per ml. One milliliter of suspension was employed per flask.

The assay for ethyl mercaptan formation, carried out in double sidearm Warburg flasks, utilized both the volatility of the mercaptan and its formation of an insoluble white precipitate, C_2H_5SHgCl, on reaction with $HgCl_2$. The main compartment of the Warburg flask contained 1.0 ml. of a buffered potassium chloride solution (20 parts 0.1 M K phosphate, pH 7.0; 40 parts 0.5 M potassium chloride; 40 parts 1.15 per cent potassium chloride), 0.5 ml. of water solution or suspension containing 5 mg. of appropriate thioethyl compound adjusted to pH 7.0, and enough 1.15 per cent potassium chloride to bring the volume to 2.5 ml. for experiments with mouse tissue, and to 1.6 ml. for experiments with bacterial suspensions.

The flasks were then placed in cracked ice, tissue or bacteria was added to the main compartment, and 0.2 ml. 1 per cent $HgCl_2$ was placed in each sidearm. The total liquid volume was 3.0 ml.

[1] From the Merck Institute for Therapeutic Research, and the Research Laboratories of the Chemical Division of Merck & Co., Inc., Rahway, New Jersey.

Incubation of the flasks, seated on manometers, was carried out at 37°C. with shaking in the conventional Warburg water bath. Ethyl mercaptan is sufficiently volatile to distill over from the main compartment into the sidearms containing $HgCl_2$, and can be detected in semiquantitative manner by the amount of precipitate formed. On visual observation at thirty-minute intervals over a three-hour incubation period, the amount of precipitate was recorded as trace, ±, or 1+ to 4+, and the rate of ethyl mercaptan formation was plotted accordingly.

With the great majority of thioethyl compounds, there was no ethyl mercaptan formation in control flasks lacking tissue or bacteria. In the case of a few compounds unstable in water solution, there was considerable release of mercaptan in such control flasks, and the data were corrected for this nonbiologic cleavage. The rate of cleavage to ethyl mercaptan is reported as the maximum amount of precipitate observed.

The antituberculous efficacy of the thioethyl compounds was evaluated (1) on the basis of inhibition of gross lung involvement. A score of

0 = no gross involvement
1 = minimal, less than 10 per cent of lung involved
2 = moderate, 10 to 25 per cent of lung involved
3 = moderate to advanced, 25 to 50 per cent of lung involved
4 = advanced, more than 50 per cent of lung involved

RESULTS

The preliminary studies on cleavage by mouse tissue were conducted with S-ethyl-L-cysteine, since it had been subjected to the most extensive therapeutic studies, and its action appeared to depend on the release of volatile ethyl mercaptan within the mouse. Despite the demonstrable cleavage of this compound by rat liver (4), no ethyl mercaptan release from this compound could be detected with mouse liver, lung, kidney, intestine, or blood; or by mouse liver under aerobic or anaerobic conditions, with or without supplementation of adenosine triphosphate and other co-factors. However, under similar experimental conditions, diethyl disulfide and various ethyl thioesters were rapidly cleaved by mouse liver or lung to ethyl mercaptan.

Further studies on the mechanism of S-ethyl cysteine cleavage disclosed that two bacterial species, freshly isolated from mouse intestinal contents, were capable of releasing ethyl mercaptan from S-ethyl cysteine at an appreciable rate. These two organisms were identified as a strain of *Escherichia coli* and one of *Streptococcus faecalis*. It was subsequently found that laboratory stock strains of these organisms could also effect the cleavage. It thus appeared that there were two mechanisms for ethyl mercaptan release from the antituberculous thioethyl compounds, only one of which involved mouse tissue.

A large number of thioethyl compounds with and without therapeutic activity was tested for rate of mercaptan liberation on incubation with mouse liver and lung and with bacterial suspensions, in order to determine whether a correlation existed between the therapeutic efficacy and the rate of mercaptan formation. Such a correlation was indeed found. The thioethyl compounds tested could be separated into three groups:

All thioethyl compounds inactive for therapy at the upper level of 0.2 per cent in diet were not significantly attacked.

Those compounds with high therapeutic activity at 0.05 per cent and 0.1 per cent in diet were rapidly cleaved to ethyl mercaptan by mouse liver and lung, and some of these were also attacked by the bacteria, particularly *S. faecalis*.

Compounds with moderate therapeutic activity at 0.1 to 0.2 per cent in diet, S-ethyl-L-cysteine and derivatives, were cleaved only by the bacteria and not at all by mouse tissue.

The data on which these groups were separated are presented in table 1 (ethyl thioesters and related compounds) and table 2 (S-ethyl-L-cysteine and

TABLE 1

ETHYL THIOESTERS AND RELATED COMPOUNDS

Compound	Lung Score at Diet Per Cent Concentration			Ethyl Mercaptan Release by			
				Liver	Lung	*E. coli*	*S. faecalis*
	0.2	0.1	0.05	Amount	Amount	Amount	Amount
Alkyl							
1 Ethyl thioacetate	0.6	—	—	1+	1+	—	—
2 Ethyl thiolaurate	0.0	0.8	4.0	1+	0	—	—
3 Ethyl thio-3,5,5-trimethyl-hexanoate	0.2	0.3	0.8	1+	1+	0	0
Aryl							
4 Ethyl thiobenzoate	0.0	0.0	1.0	4+	4+	0	±
5 Ethyl iminothiobenzoate	—	0.2	2.0	2+	2+	1+	2+*
6 Ethyl thioanisate	0.2	1.2	3.0	4+	3+	—	—
7 Ethyl (*p*-ethylsulfonyl) thio-benzoate	—	0.0	3.9	3+	3+	—	—
Heterocyclic							
8 Ethyl thiofuroate	0.0	0.0	0.4	4+	4+	0	1+
9 5-NO_2 ethyl thiofuroate	—	0.2	—	4+	4+	0	1+
10 Thiophene-2-carboxylic ethyl thioester	0.1	—	4.0	4+	4+	0	1+
11 Ethyl thioisonicotinate HCl	0.0	0.2	3.0	4+	2+	2+	4+
Carbamate							
12 S-ethyl‡thiocarbamate	3.4	—	—	0	±	—	—
13 S-ethyl di‡thiocarbamate	4.0	—	—	0	0	0	0
Carbonate							
14 O,S-Diethyl thiocarbonate	0.0	—	0.3	4+	4+	0	0
Thiosulfate							
15 Na ethylthiosulfate	—	1.1	4.0	4+	1+	0	—
Disulfides							
16 Dimethyl disulfide	—	4.0	—	4+	3+	—	—
17 Diethyl disulfide	—	0.0	0.0	4+	4+	0	0
18 Dibutyl disulfide	—	4.0	—	0	0	—	—
Miscellaneous							
19 2-Ethylmercaptobenzoxazole	—	2.3	—	0	0	0	0
20 S-ethyl thiourea-HBr	4.0	—	—	0	0	0	0
21 S-ethyl mercaptoquinoline	—	3.2	—	0	0	0	0

* Values obtained after 30 minutes' incubation. Chemical breakdown of the compound rendered further readings inaccurate.

TABLE 2

S-Ethyl Cysteine Derivatives

Number and Compound	Lung Score at Diet Per Cent Concentration			Ethyl Mercaptan Release by	
	0.2	0.1	0.05	*E. coli* Amount	*S. faecalis* Amount
22 S-ethyl-L-cysteine	0.5	2.6	2.9	1+	2+
23 S-ethyl-*N*-carboxy-L-cysteine Ca salt	0.1	3.5	4.0	1+	2+
24 S-ethyl-*N*-acetyl-DL-cysteine Na salt	3.1	3.9	4.0	0	0
25 S-ethyl-*N*-carbamido-L-cysteine	4.0	—	—	0	0
26 S-ethyl-L-cysteine sulfoxide	4.0	—	—	1+	0
27 S-ethyl-*N*-carbamido-L-cysteine sulfoxide	4.0*	—	—	0	0
28 *β*-ethyl mercapto propionic acid	—	2.6	4.0	1+	2+
29 *β*-ethyl mercapto propionamide	—	4.0	—	0	0
30 S-methyl-L-cysteine	4.0	—	—	1+	—
31 S-ethyl-L-homocysteine (ethionine)	3.4	—	—	0	0
32 L-5-ethylmercaptomethyl hydantoin	0.3	1.3	4.0	1+	1+
33 L-5-ethylsulfinylmethyl hydantoin	1.1	—	—	0	0

* 10 mg. per mouse per day administered subcutaneously.

derivatives). The thioester linkage is rapidly cleaved by mouse liver and lung, and compounds (Nos. 1–11) containing this group had high therapeutic activity. However, esterification of a carbamido rather than carboxyl group (as in Nos. 12 and 13) produced compounds inactive in both assays. It is interesting that dimethyl disulfide (No. 16) is reduced to mercaptan about as readily as diethyl disulfide (No. 17), but the former shows no therapeutic activity. Nor have any other thiomethyl compounds been effective against tuberculosis *in vivo*, although methyl mercaptan does inhibit the growth of *M. tuberculosis* H37Rv *in vitro*. The lack of activity of the thiomethyl compounds (*see also* S-methyl-L-cysteine, No. 30) is most likely due to the extremely high volatility of methyl mercaptan; even if formed rapidly within the mouse, it might escape too readily for an effective concentration to be maintained.

Only a few preliminary studies have been carried out on the thioesterase of mouse liver. Enzyme activity could be obtained in the supernatant of mouse liver homogenate centrifuged at 18,000 × g. Quantitative measurement of activity in bicarbonate buffer is complicated by the release on ester hydrolysis, not only of carbon dioxide, but also of the volatile ethyl mercaptan. However, preliminary experiments on comparative rates of hydrolysis of several thioesters presented results analogous with those obtained by the mercury precipitation method. It should be noted that, while all of the compounds can be hydrolyzed by alkali, only the enzymatically cleavable thioethyl compounds are also labile in neutral and acid solutions.

The breakdown of S-ethyl-L-cysteine by the intestinal bacteria, but not by mouse tissue, was of particular interest, since this fact offered an explanation for the finding of lower efficacy when S-ethyl-L-cysteine was given subcutaneously than when fed in the diet (5). The hypothesis that the release of ethyl mercaptan

in the mouse is dependent on intestinal organisms is strongly supported by the correlation between the therapeutic and enzymatic assays on S-ethyl cysteine derivatives presented in table 2. The only apparent exceptions are the two sulfoxides, S-ethyl-L-cysteine sulfoxide (No. 26) and L-5-ethylsulfinylmethyl hydantoin (No. 33).

S-ethyl-L-cysteine sulfoxide (No. 26) and its *N*-carbamido derivative (No. 27) were found to be reduced by *E. coli* in the presence of hydrogen; this organism has been shown previously to reduce methionine sulfoxides (6). The reduction of the sulfoxides is not carried out by *S. faecalis*, which lacks the requisite enzyme hydrogenase. The formation of mercaptan from S-ethyl-L-cysteine sulfoxide thus appears to be a two-step process: (*1*) reduction of the sulfoxide to S-ethyl-L-cysteine, and (*2*) cleavage of the sulfur-carbon bond to form ethyl mercaptan. The lack of therapeutic activity of this sulfoxide may result from inability of the reaction sequence to produce enough mercaptan in the rapid passage of intestinal contents in the mouse. Although S-ethyl-*N*-carbamido-L-cysteine sulfoxide can be reduced to S-ethyl-*N*-carbamido-L-cysteine, the latter cannot be cleaved to mercaptan, and neither of these derivatives had activity.

The activity of L-5-ethylsulfinylmethyl hydantoin in the absence of cleavage is the only significant exception to the premise of ethyl mercaptan as the active agent of thioethyl compounds. The fact that it is considerably more active therapeutically than S-ethyl-L-cysteine when administered subcutaneously suggests that it may act by another mechanism.

The mercaptan cleavage product of S-ethyl-L-cysteine has been isolated as the mercurichloride derivative, C_2H_5SHgCl (7), and identified as C_2H_5SH. Detailed studies on the reaction mechanism have not been carried out but, since the reaction occurs anaerobically, and ammonia is released concomitantly with the mercaptan, a reaction similar to cysteine desulfhydrase is suggested. However, while *E. coli* grown with cysteine attacked cysteine much more rapidly than did cells grown without it, the rate of S-ethyl cysteine breakdown by these cells was not increased. Nor did growth on S-ethyl cysteine affect the rate of cysteine breakdown. It is not yet known whether this lack of simultaneous adaptation is due to the presence of two distinct enzymes or to permeability factors. The mechanism of cleavage of S-ethyl cysteine appears to be similar to the methionine dethiomethylase of *Pseudomonas*, reported by Kallio and Larson (8).

SUMMARY

The antituberculous efficacy in mice of a series of thioethyl compounds is due to their cleavage to the active agent ethyl mercaptan. Compounds with the thioester linkage are hydrolyzed by mouse liver and lung, which also rapidly reduce diethyl disulfide. Derivatives of S-ethyl-L-cysteine are decomposed by the intestinal bacteria, *Escherichia coli* and *Streptococcus faecalis*. Thus, the antituberculous activity of this thioethyl series appears to represent a consequence of the symbiotic relationship between the mouse and its intestinal flora.

SUMARIO

Disociación Metabólica de los Compuestos Tioetílicos Antituberculosos

La eficacia antituberculosa mostrada en los ratones por una serie de compuestos tioetílicos se debe a su disociación al agente activo, el mercaptán de etilo. Los compuestos con la asociación tioestérica son hidrolizados por el hígado y el pulmón del ratón, que también reducen rapidamente el bisulfuro dietílico. Los derivados de la S-etilo-*L*-cisteína son descompuestos por las bacterias intestinales, *Escherichia coli y Streptococcus faecalis*; la actividad antituberculosa parece, pues, fundarse en una interesante relación simbiótica.

RESUME

Clivage métabolique de composés thioéthyle tuberculostatiques

L'efficacité antituberculeuse chez la souris d'une série de composés thioéthyle est due à leur clivage en éthyl mercaptan, l'agent actif. Les composés avec liaison thioester sont hydrolysés par le foie et le poumon de la souris, qui réduisent aussi rapidement le sulfure d'éthyle. Les dérivés S éthyl-L cystéine sont décomposés par les bactéries intestinales *Escherichia coli* et *Streptococcus faecalis*. L'activité tuberculostatique paraît ainsi dépendre d'une intéressante relation de symbiose.

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ENUMERATION OF VIABLE TUBERCLE BACILLI[1, 2]

MAURICE S. TARSHIS AND SANFORD S. ELBERG

With the technical assistance of Kenneth Faunce, Jr., and Dolores Mueller

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INTRODUCTION

The enumeration of viable tubercle bacilli by surface plate counts has been studied in detail by Fenner and his colleagues (1–4). An appraisal of this method on glycerol-blood-agar medium (5) seemed advisable because the medium is being used in studies on the air-borne behavior of *M. tuberculosis*. In these studies, the response of guinea pigs to air-borne infection with tubercle bacilli is being investigated in the cloud chamber described by Leif and Krueger (6). It was also deemed appropriate to compare the glycerol-blood-agar medium with other currently used media with respect to their usefulness in microbial enumerations. The other media studied were the oleic-acid–albumin–agar medium of Davis and Dubos (8) and the charcoal agar medium and charcoal water diluent of Hirsch (9, 10).

MATERIAL AND METHODS

Glycerol-blood-agar medium

Bacto-agar (Difco[3])	1.5 gm.
Glycerol (analytical grade)	1.0 ml.
Human bank blood[3]	25.0 ml.
Water (distilled)	74.0 ml.

The agar was dissolved in the glycerol-water by heating. The mixture was sterilized at 15 pounds pressure (121°C.) for fifteen minutes and allowed to cool to 45°C. The blood was added, the ingredients were thoroughly mixed, and then 10 ml. of completed medium were dispensed into sterile 50 mm. Petri dishes and allowed to harden. The final pH was 6.8.

Oleic-acid–albumin–agar medium

Basal:	
Bacto-asparagine (Difco)	2.0 gm.
Sodium phosphate, dibasic, crystals ($Na_2HPO_4 \cdot 12\ H_2O$, reagent grade)	6.3 gm.
Potassium phosphate, monobasic (KH_2PO_4, reagent grade)	1.0 gm.
NZ-amine, Type B (Sheffield)[4]	0.5 gm.
Ferric ammonium citrate (green pearls, USP grade)	0.05 gm.
Magnesium sulfate crystals ($MgSO_4 \cdot 7H_2O$, reagent grade, added as 1 ml. of a 1 per cent solution)	0.01 gm.

[1] From the Department of Bacteriology, University of California, Berkeley, California.

[2] This study was aided by a grant from the Alameda County, California, Tuberculosis Association.

[3] Outdated bank blood approximately four weeks old was used and contained ACD solution as follows: citric acid, 0.48 gm.; trisodium citrate, 1.32 gm.; and dextrose, 1.47 gm. per each 100.0 ml. of transfusion solution. Approximately 500 ml. of blood were collected in 120 ml. of ACD solution. To obviate possible inhibitory effects of any single blood, although such a problem has not been observed, several specimens of the same type were pooled before use.

[4] Sheffield Farms Co., Inc., New York, New York.

Calcium chloride ($CaCl_2$, reagent grade, added as 1 ml. of a 0.05 per cent solution)	0.0005 gm.
Zinc sulfate ($ZnSO_4$, reagent grade, added as 1 ml. of a 0.01 per cent solution)	0.0001 gm.
Copper sulfate ($CuSO_4$, reagent grade, added as 1 ml. of a 0.01 per cent solution)	0.0001 gm.
Water (distilled)	900.0 ml.
Bacto-agar (Difco)	15.0 gm.

The mixture was heated to dissolve the ingredients, then cooled to room temperature, adjusted to pH 6.6, and sterilized at 15 pounds pressure (121°C.) for fifteen minutes.

Oleic-acid–albumin complex

Oleic acid (USP, grade)	0.12 ml.
Sodium hydroxide (N/20)	10.0 ml.
Bovine albumin[5]	95.0 ml.

By means of shaking with a rotary motion, the oleic acid was dissolved in a small flask containing the sodium hydroxide. Then 5.0 ml. of this mixture were added to the albumin and the solution was sterilized by Seitz filtration.

Completed medium: To 900.0 ml. of the basal medium at 45°C. were added 100.0 ml. of the oleic-acid–albumin complex. The medium was dispensed in 10 ml. amounts into sterile 50 mm. Petri dishes and allowed to harden. The final pH was 7.1.

Charcoal-agar medium

Bacto-asparagine (Difco)	2.0 gm.
Ferric ammonium citrate (brown pearls, USP grade)	0.1 gm.
Glycerol (reagent grade)	10.0 ml.
Potassium phosphate, monobasic (KH_2PO_4, reagent grade)	4.0 gm.
Sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$, reagent grade)	1.0 gm.
Sodium phosphate, dibasic, crystals ($Na_2HPO_4 \cdot 12H_2O$, reagent grade)	3.0 gm.
Magnesium sulfate, crystals ($MgSO_4 \cdot 7H_2O$, reagent grade)	0.1 gm.
Bacto-tryptone (Difco)	5.0 gm.
Water (distilled)	1,000.0 ml.

The mixture was dissolved by heating gently, and then 10.0 gm. of agar-agar (granular)[6] were added, followed by the addition of 1.0 gm. of Norit A.[7] The mixture was stirred well, and to it were added 25.0 ml. of a 1 per cent solution of cholesterol in acetone. After mixture, 250 ml. amounts were dispensed in 500 ml. Erlenmeyer flasks with nonabsorbent cotton plugs, placed in flowing steam for five minutes to drive off acetone, and then sterilized at 15 pounds pressure (121°C.) for fifteen minutes. After slow release of pressure, the medium was again mixed well, and 10 ml. amounts were placed into sterile 50 mm. Petri dishes and allowed to harden. The final pH was 7.0.

All media were incubated at 37°C. for forty-eight hours and checked for sterility before use.

The H37Rv strain of *M. tuberculosis* var. *hominis* was grown in Tween®-albumin liquid medium[8] for seven days, after which time serial dilutions of the culture were prepared in both 0.01 per cent charcoal water[9] (10) and 0.1 per cent aqueous crystallized bovine plasma albumin.[10] The drop method of inoculating the plates was based upon a slight modification of the method of Miles and Misra (11), in which an 18-gauge needle was fitted to a glass

[5] The Armour Laboratories, Chicago, Illinois. Control No. 11101 (Fraction V); prepared as a 5 per cent neutral solution in 0.85 per cent sodium chloride.

[6] Baltimore Biological Laboratory.

[7] Decolorizing Carbon, Pfanstiel Chemical Co., Waukegan, Illinois.

[8] This medium was prepared exactly as described for the oleic-acid–albumin–agar medium, deleting the agar and adding Tween 80 in 0.02 per cent and bovine plasma albumin (Fraction V) in 0.5 per cent final concentrations. The final pH was 6.8.

TABLE 1

AVERAGE NUMBER OF BACTERIAL COLONIES PER PLATE
(20 Replicates)

Trial	Plating Medium	Diluent					
		Albumin			Charcoal		
		10^{-3}	6×10^{-4}	10^{-4}	10^{-3}	6×10^{-4}	10^{-4}
A.M.	Blood	25.05	13.50	3.00	22.95	13.00	2.45
	Charcoal	20.25	10.10	1.75	18.05	9.95	2.35
	Oleic acid	24.40	11.80	1.90	17.35	12.00	1.85
P.M.	Blood	27.70	20.45	3.40	16.70	12.35	2.65
	Charcoal	22.60	16.65	2.00	14.35	9.30	1.75
	Oleic acid	20.50	16.65	2.75	14.45	9.80	1.45

tube with a rubber bulb. Ten plates of each medium were mixed randomly and inoculated with two drops of culture from each of three dilutions for each diluent fluid, using a separate needle for each dilution. Every needle was calibrated for each diluent to determine the number of drops delivered per ml.

Since there was considerable variation in the number of drops delivered, according to the angle at which the needles were held, the variations were minimized by bending the tip of the needle in such a way that delivery of the drops could be made in a vertical position with the tip held approximately 1 cm. from the surface of the medium.

After the plates were inoculated, they were allowed to stand undisturbed until all the drops of inocula were absorbed; they were then placed in plastic bags, which were sealed with scotch tape and incubated at 37°C. Readings of the plates were made daily under the binocular stereoscopic microscope at a magnification of 9×, using incident illumination, and the day on which growth first appeared on each medium was noted. Final colony counts on all of the media were arbitrarily made after eighteen days. After this time the coalescence of colonies, particularly on the blood and oleic-acid–agar media, increased, and this not only made counting difficult, but gave lower counts.

RESULTS

A representative set of experiments is presented in table 1, in which the procedure was carried out *ab initio* in both the morning and afternoon of this particular trial. The table presents the actual means of the 20 replicate counts for each combination studied. Although the same stock suspension was employed for both the morning and afternoon trials, a higher mean count was consistently obtained on the suspension in the afternoon series. No explanation for this is at hand other than differences in the skill of the two teams.

In table 2 is presented the square root transformation of the data in table 1 with the standard deviations in preparation for the analyses of variance.

The greater reproducibility of the values obtained in the combination blood-agar-albumin diluent is clearly evident from the standard deviation and this is closely followed by the results on the combinations of blood-agar-charcoal

[9] This is the same product that was used in the preparation of the charcoal agar medium. It was sterilized at 15 pounds (121°C.) for fifteen minutes.

[10] The Armour Laboratories, Chicago, Illinois. Lot No. P67502. The albumin solution was sterilized by Seitz filtration.

TABLE 2

SQUARE ROOT TRANSFORMATION WITH STANDARD DEVIATION
(From Data in Table 1)

			Diluent					
			Albumin			Charcoal		
			10^{-5}	6×10^{-5}	10^{-4}	10^{-5}	6×10^{-5}	10^{-4}
Means	A.M.	Blood	4.99	3.63	1.69	4.77	3.57	1.49
		Charcoal	4.47	3.09	1.08	4.19	3.12	1.41
		Oleic acid	4.92	3.49	1.31	4.10	3.44	1.26
	P.M.	Blood	5.25	4.50	1.80	4.06	3.48	1.54
		Charcoal	4.78	4.04	1.29	3.76	3.02	1.21
		Oleic acid	4.50	4.04	1.61	3.78	3.10	1.08
Standard Deviations	A.M.	Blood	.38	.53	.39	.47	.49	.46
		Charcoal	.48	.76	.85	.72	.48	.69
		Oleic acid	.49	.46	.44	.73	.35	.52
	P.M.	Blood	.37	.44	.40	.47	.46	.52
		Charcoal	.50	.59	.58	.42	.40	.54
		Oleic acid	.59	.58	.39	.43	.47	.54

diluent and oleic-acid–charcoal diluent in the afternoon series. This series was in general a much more reproducible one than the "albumin diluent series" carried out at the same time.

Departures from proportionality in the dilution series occurred consistently on the low side in the morning series and on the high side in the afternoon series with both diluents in the 6×10^{-5} dilution.

For each plating medium the albumin diluent gave a higher value than the charcoal diluent at all dilutions, with only two exceptions.

In the analyses of variance, the study was treated as twelve experiments in which three plating media were each studied with two diluting fluids, at three dilution levels, on each of two occasions. The F test revealed (table 3) that on ten of the twelve occasions the count on the blood agar was significantly higher at the 5 per cent level than the count on either of the other two plating media.

Using a method of multiple contrasts developed by Scheffé in the University's Institute of Mathematical Statistics, the contrast between blood agar and charcoal agar revealed a significantly higher count on the blood agar in 9 of the 12 occasions, and a significantly higher count on the blood agar over that on oleic-acid–agar on six of the twelve occasions. When twice the value on blood agar was contrasted with the sum of the counts on the other two media, in order to achieve a comparison of the one medium to both of the other simultaneously, the count on blood agar was significantly higher on seven of the twelve occasions.

Although the analyses of variance could have been extended to make many other comparisons, it seems more in order at this point to emphasize that the differences obtained with any *one* medium, when the experiments in the morning are contrasted with those in the afternoon, were of the same magnitude as were observed

TABLE 3

ANALYSIS OF VARIANCE

Comparison of the Effects of Three Plating Media in 12 Separate Experiments with Transformed Plate Counts

	Albumin-AM			Albumin-PM			Charcoal-AM			Charcoal-PM		
	10^{-5}	5×10^{-5}	10^{-6}	10^{-5}	5×10^{-5}	10^{-6}	10^{-5}	5×10^{-5}	10^{-6}	10^{-5}	5×10^{-5}	10^{-6}
Blood	99.79	72.68	33.77	105.00	89.99	36.01	95.35	71.45	29.85	81.20	69.68	30.82
Charcoal	89.49	61.73	29.15	94.55	80.74	25.74	83.74	62.34	28.24	75.29	60.47	24.17
Oleic acid	98.30	68.09	26.15	89.85	80.79	32.25	82.01	68.93	25.10	75.54	61.91	21.54
F2, 57	7.04	3.98	11.65	12.00	4.58	5.87	5.85	5.29	1.00	2.80	5.86	3.86
P	0.005	0.025	0.005	0.005	0.025	0.005	0.010	0.001	———	0.100	0.005	0.050

with the *different* media studied at the same time. Thus the differences observed between the media are *within* the range of precision of the viable count method as it was performed in this study. If the study indicates anything remarkable, it is the very great similarity of the results obtained by the technique used here. Furthermore, advantages which seem to accrue to any one set of experimental conditions were perhaps reduced in effectiveness by the differences which occurred in the morning as contrasted with the afternoon series. This effect of the "time parameter" could conceivably be reduced by increasing competence of the investigators and offers some confirmation to the view that for this type of work standardization of the human element is desirable.

Although counts could be made on the blood medium in ten days, the eighteen-day period was arbitrarily chosen for final counts for several reasons. First, the increase in the number of colonies from fourteen to eighteen days was considerably less than that from ten to fourteen days. Thus, of 30 counts employing albumin diluent and glycerol-blood agar, 24 changed during the ten- to fourteen-day period, whereas only 2 changed during the fourteen- to eighteen-day period. In the combination charcoal diluent—glycerol-blood agar, 23 of 30 counts were altered during the ten- to fourteen-day period. The same pattern was observed in the other combinations of diluent fluids and plating media.

Second, when using the charcoal diluent and the blood medium, it was not always possible to distinguish sufficiently clearly at ten days between early growth and the charcoal particles which appeared under the stereoscopic microscope as shiny refractile bodies simulating the tiny silvery gray pin-point colonies of tubercle bacilli. In many instances, however, colonies were sufficiently large by this time and thus did not present such a problem. This difficulty was not encountered with the albumin diluent, so that preliminary estimates of viable bacilli could be made as early as two days on the blood medium, depending upon the size of the inoculum.

Finally, since viable growth appeared somewhat more slowly on both the oleic-acid–albumin and the charcoal-agar media, counting much before this time would have been more difficult on these media, and certainly the results would have been less accurate. Since the increase in the number of colonies from ten- to fourteen-day periods was so slight on the blood medium using the

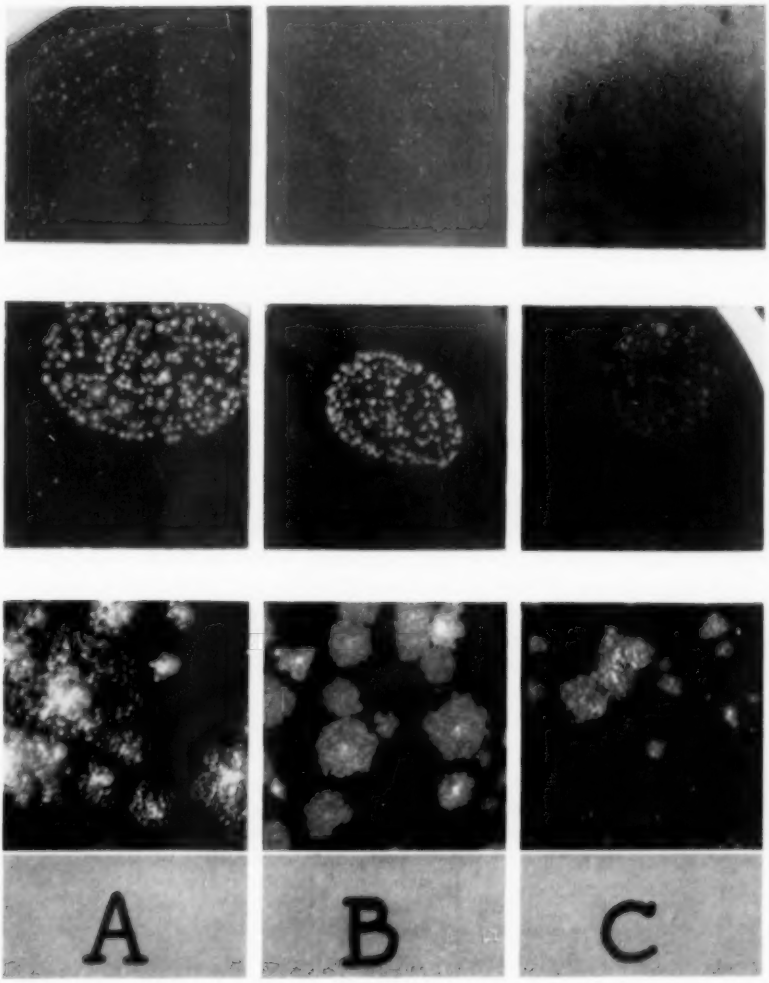

FIG. 1. (Top) Comparison of growth of the H37Rv strain of *M. tuberculosis* on (*a*) glycerol blood agar, (*b*) oleic-acid–albumin, and (*c*) charcoal agar media after ten days at 37° C. (×1.6) *Inoculum:* Single drops from calibrated needle of a 10^{-4} dilution in 0.1 per cent crystallized bovine plasma albumin of a seven-day-old culture in Tween-albumin liquid medium.

(Middle) Same as top, but after fourteen days' incubation. (×1.7)

(Bottom) Photomicrographs of colonies in middle section to demonstrate morphology on different media. (×15.5) Precipitated matter in charcoal medium, appearing as grayish-white plaques, is probably cholesterol. This makes detection of early growth difficult.

albumin diluent, the earlier period could have been used for making final counts without significant error.

DISCUSSION

In the study presented here a simple and reliable technique for the enumeration of viable tubercle bacilli is described. It has also been demonstrated that the glycerol-blood-agar medium and the bovine plasma-albumin diluent can be used very satisfactorily for this purpose. Of interest are the results reported by Fenner (1) in which he found the oleic-acid–albumin–agar medium and 0.1 per cent bovine plasma-albumin diluent more satisfactory for viability determinations than the Löwenstein medium and the M/15 disodium hydrogen phosphate diluent. Of interest also is the recent report by Knox (12), who pointed out that the semi-solid Kirschner as well as the Löwenstein-Jensen media compared favorably with glycerol-blood agar for viable count determinations.

The blood medium proved best for the detection of early growth because of the marked color contrast between the clear red background and the silvery gray colonies. Because of this, the observer was apt to conclude that this medium supported earlier growth, although verification of this would require a more thorough use of different systems of illumination of the colonies. This was not done in the present study. During the early periods, growth on the blood medium is more luxuriant. The types of virulent human tubercle bacilli colonies which have thus far been observed on the blood medium and their characteristic developmental changes have been described in detail previously (5, 13). On oleic-acid–albumin medium, the rate of growth was no faster than on the charcoal medium, but the size of the younger colonies was larger. Detection of early growth was also much easier on the former medium because of its clarity. The colonies on both the oleic-acid–albumin and charcoal media are very similar, growing much flatter than on the blood medium, and for this reason there is a greater coalescence of the colonies (figure 1). This makes counting more difficult when the number of colonies is large or when counts are made after coalescence occurs. The more prominent pyramidal and "nipple" type of colonies which grow on the blood medium make counting much easier when growth is heavy or after coalescence has occurred.

SUMMARY

It has been demonstrated that a method for enumerating viable tubercle bacilli on glycerol-blood agar, oleic-acid–albumin and charcoal agar, using bovine plasma-albumin diluent, gave differences among the media within the precision of the method. Ultimate selection of the blood medium for further studies was guided by its economy, simplicity of preparation, and ability to grow tubercle bacilli from small inocula recognizably in a short time.

SUMARIO

Enumeración de los Bacilos Tuberculosos Viables

Queda demostrado que un método destinado a enumerar los bacilos tuberculosos viables en agar-sangre glicerol, albúmina ácido oleico y agar-carbón vegetal, usando plasma bo-

vino-albúmina como diluente, acusó diferencias entre los distintos medios dentro de la precisión del método. La selección final del medio de sangre para estudios ulteriores se rigió por su economía, sencilla preparación y capacidad para hacer crecer de pequeños inóculos bacilos tuberculosos reconocibles al poco tiempo.

RESUME

Numération de bacilles tuberculeux viables

Il a été démontré qu'une méthode pour la numération des bacilles tuberculeux viables cultivés sur des milieux gélose-sang glycérol, acide oléique-albumine et gélose-charbon, en utilisant comme diluant l'albumine plasmatique bovine a permis d'observer des différences entre les milieux, dans les conditions de précision de la méthode. Le choix ultime du milieu au sang pour utilisation dans des études plus complètes est guidé par des raisons d'économie, de simplicité de préparation et par la possibilité de faire pousser des bacilles tuberculeux identifiables à partir d'un inoculum réduit.

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Case Reports

BRONCHOGENIC CYSTIC DISEASE COMPLICATED BY UNSUSPECTED CHOLERAESUIS AND ASPERGILLUS INFESTATION[1]

RAYMOND F. CORPE AND JEROME A. COPE

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INTRODUCTION

Various types of pulmonary complications due to the salmonella organisms have been described in the literature. Minor and White (1) noted that several early French writers wrote of the pulmonary complications of typhoid fever. Typhoid organisms were obtained on sputum examination and lung aspirate by Sahli, Jehle, Roque, and Bancel (2), among others.

Pulmonary infection due to, or at least accompanying, typhoid fever has been reported. Minor and White believe that lung abscess, due to the typhoid organisms, is the rarest of pulmonary complications. Pleural complications of typhoid infection apparently vary with the epidemic and the diligence of the medical observer. Effusions at some point in the disease process are not exceedingly rare. Empyema, however, is reported to be quite rare. Most empyemas appear to arise from a pre-existing effusion. Occasionally, typhoid osteitis or subdiaphragmatic abscess may be the etiologic factor in the subsequent empyema. Pulmonary infections of the salmonella group of organisms are found to be rather rare. Bullowa (3) was the first to report a case of pneumonia in which the infecting organism was *Salmonella choleraesuis*. Edwards and co-workers (4) reported salmonella obtained from pleural fluid in one case and from empyema in four cases. Cohen, Fink, and Gray (5), in a review of the literature, were able to find thirty-nine cases of *Salmonella choleraesuis* bacteremia, among which were six cases of pleuropulmonary disease.

There are three generally recognized clinical types of salmonellosis. The first is the gastroenteric form, manifested by diarrhea, fever, abdominal pain, vomiting, and headache. The second is a typhoid-like syndrome with fever, malaise, leukopenia, and brachycardia. The third type is the protracted bacteremia type with various manifestations and high mortality. This form is characterized by marked invasiveness and tends to localize in the lungs, bones, and joints, and most frequently is due to the *Salmonella choleraesuis*.

Aspergillosis is not a truly rare infection in the lung. Surgical excision for this lesion is apparently rare. Yesner and Hurwitz (6), in 1950, reported the third case treated by excisional surgery.

It is believed that the present case of choleraesuis and aspergillum, in combination in cystic disease of the lung, is the first such report.

CASE REPORT

C.W.J., (Case No. 23184, Battey State Hospital) a 50-year-old white male, a lumber inspector, was admitted to the hospital on July 9, 1954. The onset of the present illness dated back approximately two years prior to admission. At that time, in a routine survey film, the patient was noted to have roentgenographic evidence of pulmonary disease. He was seen, periodically, for six months, and then lost to follow-up. Sputum examinations at the

[1] From the Battey State Hospital, Rome, Georgia; and the Medical College of Georgia, Augusta, Georgia.

time of observation were reported negative for acid-fast bacilli. In the succeeding interval he apparently did fairly well and remained asymptomatic. In May, 1954, however, the patient had hemoptysis of moderate degree, with some bloody streaking which persisted for two and one-half days. Following this, he consulted his physician. A chest roentgenogram obtained at that time revealed pulmonary disease in the right upper lung field. After reviewing this film, his physician advised him to apply to the State Tuberculosis Sanatorium for admission.

Physical examination on admission was essentially negative, with the exception of the lungs, which revealed a few fine rales in the upper portion of both lung fields. The patient did not appear acutely ill.

Laboratory data: Urinalysis and the hemoglobin values were within normal limits. Routine agglutination studies for typhoid, typhus, brucellosis, and tularemia were negative. Direct smear, culture, and animal inoculation of sputum for tubercle bacilli were also negative. Skin tests for tuberculosis, blastomycosis, coccidioidomycosis, and histoplasmosis were likewise negative. Papanicolaou studies of sputum were suggestive of neoplastic process. A roentgenogram of the chest on admission revealed areas of infiltration in the right upper lung field, with areas of rarefaction (figure 1). The lateral film revealed a solid-appearing lesion (figure 2). Bronchoscopy revealed normal findings.

As bronchogenic carcinoma could not be excluded as a possibility, surgical exploration was considered mandatory. Preoperatively, the presence of what was thought to be cystic disease was also recognized (figure 3).

Operation: Surgical exploration on August 28, 1954, by one of the writers (RFC) revealed cystic areas in the posterior and apical segments of the right upper lobe (figure 4), some of which were air containing and some of which were filled with cheesy debris. The cystic areas, peripherally located, did not occupy more than 25 per cent of the total volume of the apical and the posterior segments. It was believed that these cystic areas could be excised locally in a retrograde manner, thereby conserving considerable lung tissue, which, in turn, would be of aid in space obliteration with functioning lung tissue. It was likewise believed that neoplastic disease was not present. The medial cyst, filled with cheesy material, had several communicating tertiary bronchi, which were dilated and, in removing this particular cyst, very small portions of the posterior cyst wall were left *in situ*. The contents were removed, without gross evidence of pleural contamination, along with the remainder of the cyst wall. Air leaks were combated, and it was believed that air leakage of any major degree was absent at the time of closure. The operative diagnosis was an undiagnosed type of cystic disease, probably bronchogenic in origin, with cysts secondarily filled with debris of unknown etiology.

Postoperative course: The immediate postoperative course was essentially satisfactory. However, the patient developed a persistent air leak which increased in magnitude during the next several days. The patient was uncooperative in coughing and deep breathing, and thus required several tracheal aspirations. The temperature, after the first few days, varied between 100° and 103° F. for approximately fourteen days. In the postoperative period, the patient developed auricular fibrillation, which responded to appropriate therapy. Three days postoperatively, the patient had frank purulent drainage from the thoracotomy tubes of a thin, reddish-brown material.

One week postoperatively, the bacteriologic reports on the resected material became available and a gram-positive bacillus was isolated from the cyst wall which was identified as *Salmonella choleraesuis*. In addition, *Aspergillus fumigatus* was isolated from the cyst contents (figure 5). The culture of the empyema drainage was positive for *Salmonella choleraesuis*. The sputum subsequently became positive for salmonella.

Chloramphenicol was started after drug-susceptibility studies revealed the organisms to be susceptible to this drug.

Following the institution of this therapy, a gradual clinical improvement was noted. The patient became afebrile after approximately two weeks and appeared to be improving quite rapidly. He subsequently developed a bronchopleural cutaneous fistula with a drainage of

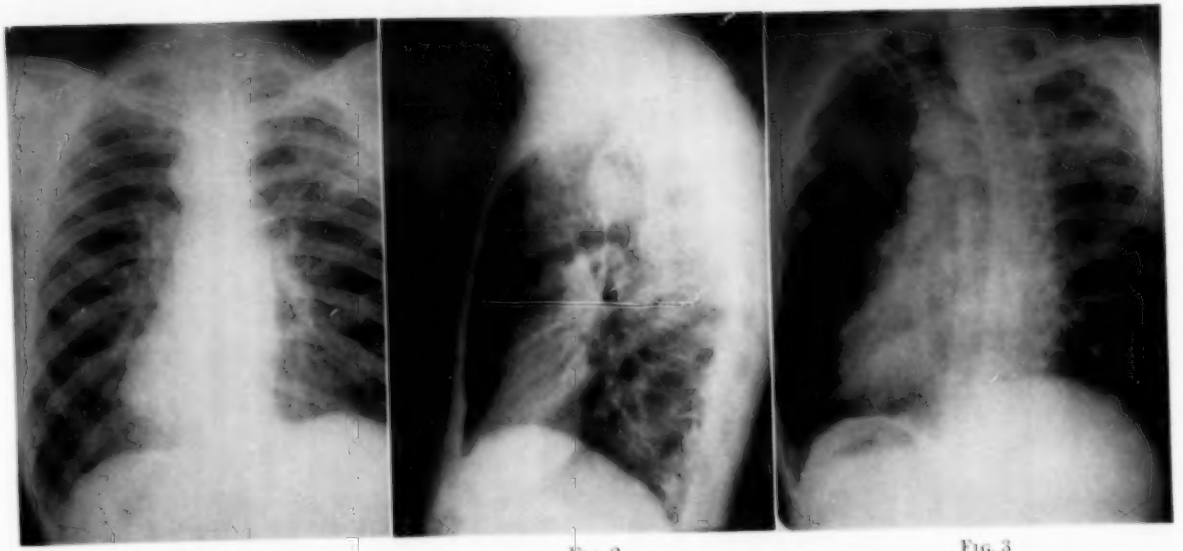

FIG. 1 FIG. 2 FIG. 3

FIG. 1 (Left). Admission chest roentgenogram (postero-anterior), showing infiltration in right upper lung field.
FIG. 2 (Center). Lateral film showing solid lesion in upper lung field.
FIG. 3 (Right). Right oblique view illustrating areas of rarefaction.

FIG. 4. Photograph of operative findings demonstrating cystic disease of right upper lobe.

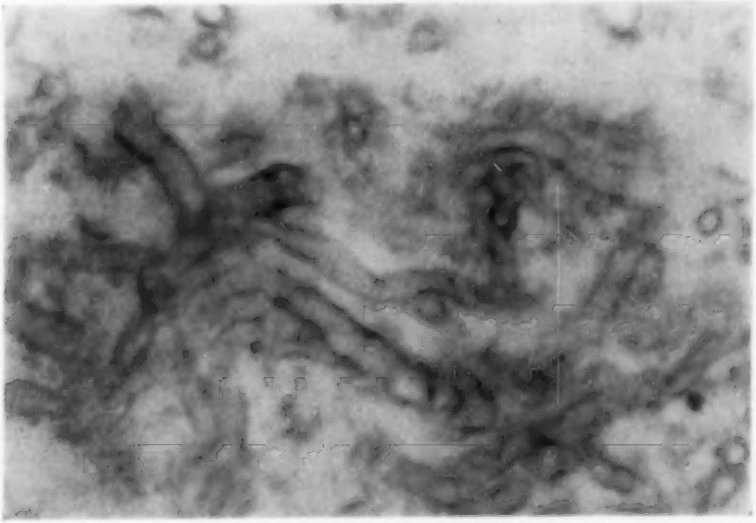

FIG. 5. Photomicrograph demonstrating aspergillus organisms obtained on smear from cyst contents.

the same brownish-red material, which was positive for salmonella. The postoperative chest roentgenograms showed continued improvement in that the lung was slowly expanding and obliterating the empyema space. The bronchopleural fistula gradually closed and remained closed, without any further therapy. The feces, in the postoperative period, remained persistently negative for salmonella. Repeated urine and blood cultures, likewise, were nega-

FIG. 6. Chest roentgenogram on discharge from hospital.

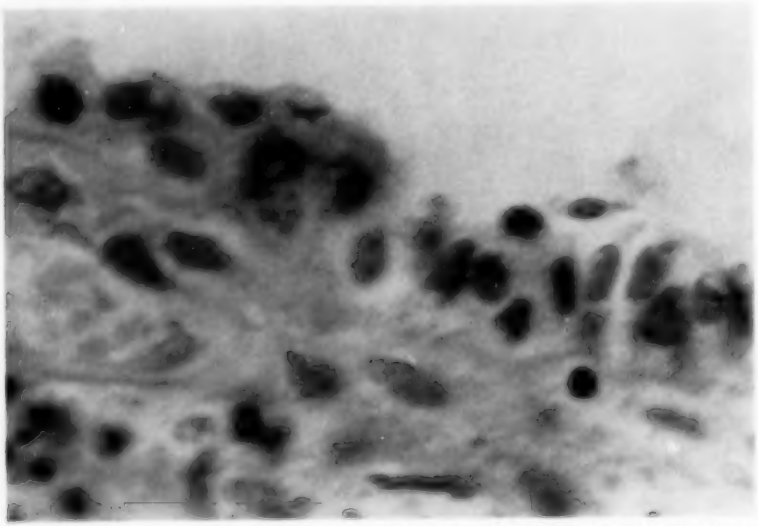

FIG. 7. High power magnification of cyst wall demonstrating bronchial epithelial lining.

dive. The salmonella agglutinations in the postoperative period became positive with a titer of 1:1,280. The patient was discharged from the hospital on November 10, 1954, afebrile and asymptomatic and with the bronchopleural fistula closed (figure 6).

The pathology report on the resected material was pulmonary cyst, bronchogenic in origin, and chronic bronchiectasis (figure 7).

COMMENT

This case presents several interesting features. The first is the isolation of *Salmonella choleraesuis* from an essentially asymptomatic patient, with known roentgenographic evidence of pulmonary disease for at least two years. The sputum, preoperatively, was repeatedly negative for salmonella. It is likewise interesting to note that the patient had a negative agglutination preoperatively for the paratyphoid group of organisms. This negative agglutination was obtained in this hospital and also in the Communicable Disease Center Laboratory in Atlanta, Georgia. Postoperatively, after the salmonella was found in the resected specimens, and subsequently in the empyema fluid, the serum agglutination became positive in a titer of 1:1,280.

Further questioning of the patient postoperatively revealed that he had worked as a farmer for many years, although not for the past eleven years. He stated that during that time he had raised hogs and was well aware of the fact that several of his animals had died of "hog cholera." He stated that, to the best of his knowledge, he was never ill at that time. He did have an episode of "pneumonia" approximately seven years ago, from which he recovered uneventfully.

The interesting feature for speculation in this case is the source of the choleraesuis organisms. An individual can harbor salmonella organisms and yet remain asymptomatic and have no serum agglutinins. Weil and Saphra (7) state that one would suspect that in salmonella carriers the continual presence of the organisms would be conducive to the maintenance of antibody output. Accordingly, a considerable amount of work has been dedicated to investigating the possibilities of the Widal test in a clinically healthy population as an indicator of carriers. Actual experience has not borne out the working hypothesis. The disappearance of antibodies from the circulation after infection occurs regardless of whether or not the infecting organisms remain in a commensal capacity. Did the patient contract his original salmonella infection at the time in his life when he was a farmer and harbor it in a congenital cyst of the lung for many years? Eisenberg and associates (8) state that, whereas five patients (5.3 per cent) in their series of 95 cases were classified as salmonella carriers, no cases of carriers of *Salmonella choleraesuis* were encountered, a circumstance that is general experience. Was the patient's pneumonia, approximately seven years prior to admission, a salmonella pneumonitis at that time? All of these questions are difficult to answer and were made even more difficult by the presence of the negative agglutination studies.

It is not believed that this patient contracted his salmonella infection in the hospital, although he was in the hospital one month prior to surgery; however, during that time he was not ill. There has never been a known case of salmonella infection in this hospital prior to the present case to serve as a possible source of infection.

The present writers prefer to accept the thesis that the patient had harbored *Salmonella choleraesuis* in the pulmonary cysts for many years without their causing any symptoms, and then, after subsequent surgery on the harboring cyst, he developed symptomatic salmonella infection. Salmonella organisms vary appreciably from strain to strain in terms of drug susceptibility. This particular strain of *Salmonella choleraesuis* was susceptible to chloramphenicol, chlortetracycline, and oxytetracycline. It was decided to use chloramphenicol and there was a favorable clinical response on the part of the patient after this therapy was instituted.

It is also interesting to speculate on the possible postoperative course in this patient if a complete right upper lobectomy had been done rather than a local retrograde excision of the cystic areas. Probably by performing a complete lobectomy the empyema and subsequent stormy postoperative course could have been avoided and, in retrospect, this would have been the preferable treatment. It is believed, however, that local excision of cystic areas is indicated in an attempt to preserve all of the lung tissue possible to aid in space obliteration, even though this case was complicated by an unusual infection.

SUMMARY

A case of congenital cystic disease of the lung, complicated by choleraesuis and aspergillum, with subsequent empyema, is presented. The possible source of this infection is discussed and suggestions concerning the operative management of cystic disease are made.

SUMARIO

Afección Quística Broncógena Complicada por Insospechadas Salmonelosis y Aspergilosis

Se presenta aquí un caso de afección quística congénita del pulmón, complicada por infestación con *S. choleraesuis* y *Aspergillus fumigatus* con empiema subsiguiente. Discútese la posible procedencia de esta infestación, ofreciéndose indicaciones acerca de la asistencia operatoria de la enfermedad quística.

RESUME

Maladie kystique bronchogène compliquée d'infection inapparente par le cholerae suis et l'aspergillus fumigatus

Présentation d'une observation de maladie kystique du poumon compliquée d'infection par le *cholerae suis* et l'*aspergillus fumigatus* avec empyème secondaire. La source possible de cette infection est discutée et les auteurs font des suggestions quant au mode de traitement chirurgical de la maladie kystique.

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TUBERCULOSIS OF THE MYOCARDIUM[1]

HAROLD L. STEIN

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The first case of myocardial tuberculosis in the English literature was described by Townsend of Dublin in 1832 (1). Involvement of the myocardium is an uncommon complication of tuberculosis. Several writers (2, 3) who have reported large series of autopsies have found the incidence of myocardial tuberculosis in tuberculous patients to be 0.24 and 0.28 per cent. Tuberculous pericarditis, however, was found in 4.4 per cent of these patients at autopsy. In tuberculous children the incidence was found to be much higher: in one series, 37 cases of tuberculous involvement of the myocardium were found in 973 autopsies (4), an incidence of 3.9 per cent. Anders (5), in his review of the world literature in 1902, found only 72 recorded cases of myocardial tuberculosis. There was no sex predilection.

The following case is reported because of the rarity of this condition and because it demonstrates the lack of cardiac symptoms in spite of extensive involvement of the heart with tuberculosis.

CASE REPORT

W.L., a 62-year-old white special police officer, was admitted to the hospital on May 2, 1953, with a chief complaint of pain in the lower back of three months' duration. The patient was well until 1941 when he was first discovered by his private physician to have hypertension. In June, 1942, he noted dyspnea. His physician diagnosed a myocardial infarction and confined the patient to bed for four weeks. After this episode the patient worked part time and never had any cardiac or pulmonary symptoms. In January, 1953, he noted the insidious onset of low back pain which was treated by application of heat to the area. In March, 1953, the patient was hospitalized in another institution, where he was fitted with a lower trunk cast for a short period. In spite of these measures, the pain progressed and the patient lost 10 pounds. He had no cough, hemoptysis, or dyspnea.

Physical examination revealed a white male, weighing 180 pounds, who appeared chronically ill. Blood pressure was 190 systolic and 130 diastolic in mm. of mercury. The left lobe of the thyroid was enlarged and it extended behind the sternomastoid muscle. It was stony hard, fixed to the surrounding deep structures, fairly well circumscribed, and approximately 2 inches in diameter. A few fine rales were present in the base of the left lung. The heart was normal.

Hospital course: An electrocardiogram obtained on May 4, 1953, revealed a low T wave and the ST segment slightly depressed in leads 1, 5, and 6. These changes were consistent with "left ventricular strain" pattern. A roentgenogram obtained on May 4, 1953, revealed no evidence of infiltrate in either lung. The heart was enlarged to the left. A large paratracheal mass was noted on the right. A roentgenographic survey of the bones disclosed a destructive process involving the lower portion of the eleventh dorsal and sixth and seventh cervical vertebrae. On May 22, 1953, a total thyroidectomy was done. The left lobe of the thyroid was enlarged and necrotic. A 2 cm. firm partially calcified nodule was present in the right lobe. Microscopic examination of the thyroid revealed necrotic adenocarcinoma with tubercle formation in one area which was negative for acid fast bacilli on direct stain. Postoperatively, the patient developed hypoparathyroidism that responded to treatment. He continued to complain about low back pain. The presumptive diagnosis was metastatic

[1] From the Laboratory and Surgical Services of the Veterans Administration Hospital, Bronx, New York.

carcinoma to the vertebrae which was treated with a total depth dose of 2,530 roentgens in fifteen treatments over a period of twenty-two days. A roentgenogram on June 25, 1953, revealed extensive nodular infiltrates throughout both lungs. The etiology was unknown, but was thought to represent a lymphangitic spread of tumor.

Another examination on June 26, 1953, revealed a more pronounced reticular infiltrate in both lungs and enlargement of both hilar regions. On September 9, 1953, the patient developed projectile vomiting, associated with mild nystagmus and lateral deviation of the left eye and weakness of the left upper extremity. Bilateral Babinski reflexes were present. Lumbar puncture produced bloody fluid under 320 mm. water pressure. The patient lapsed into semicoma and developed nuchal rigidity. His temperature rose to 101°F., and he became progressively worse. He died on October 25, 1953.

Necropsy: On gross examination, the body was that of a cachetic 62-year-old white male with a well-healed surgical scar over the anterior portion of the neck. The right pleural cavity was almost obliterated by dense adhesions; but in the lower portion 300 ml. of amber-colored fluid was present. The left pleural cavity contained 156 ml. of similar fluid, but no adhesions. The pericardial cavity was lined by smooth, glistening membrane, and contained approximately 100 ml. of amber fluid.

The heart weighed 420 gm. and the left ventricle was hypertrophied. At its thickest portion the left ventricle measured 2 cm. and the right ventricle, 1 cm. Starting the incision in the aorta and cutting down into the left ventricle close to the septum, an area of necrosis

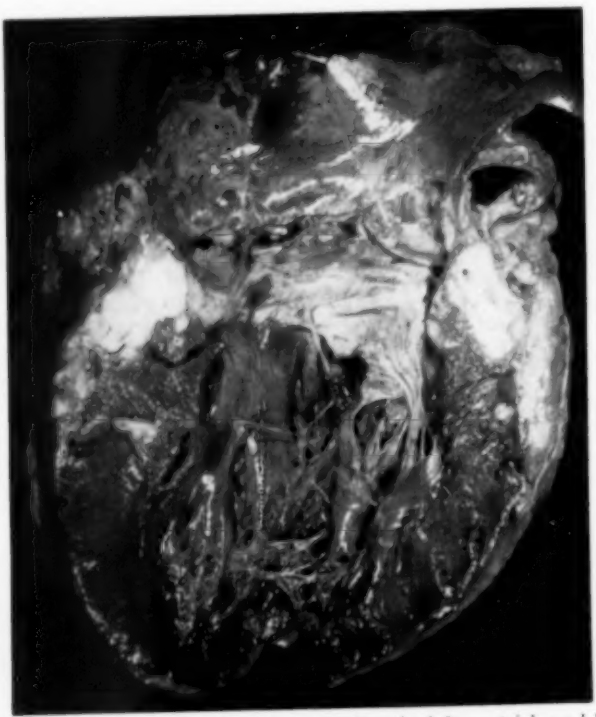

FIG. 1. Heart transected from the aorta, exposing the left ventricle and bisecting the caseous lesions surrounding the coronary ostia.

in the myocardium was entered. This was filled with a yellow-white, thick material just below the aortic valve leaflets and the coronary ostia (figure 1) and measured 2 by 1.5 by 3 cm.

The coronary arteries, although thickened, were patent throughout. The endocardium was smooth without evidence of ulceration or thrombosis. The mitral valve leaflets were firm, calcific, and admitted one finger. The aortic, tricuspid, and pulmonic valves were normal. Considerable atherosclerosis was present in the aorta. The pulmonary vessels were normal.

Both lungs were studded with small, firm, white nodules measuring 3 mm. each. A firm mass of lymph nodes measuring 4 by 3 by 4 cm. was present at the carina, as well as numerous paratracheal and parahilar nodes. The gastrointestinal tract, liver, and kidneys were grossly normal. The spleen and adrenals contained many firm, white nodules. There was no evidence of recurrent tumor in the neck. The fifth lumbar vertebra was soft and collapsed. Examination of the brain revealed moderate arteriosclerosis with recent hemorrhage into the right caudate nucleus and internal capsule with partial rupture into the right lateral ventricle. At the depth of the right post-central sulcus a circumscribed, firm white area was present in the cortex and subcortical white matter. There was no apparent primary focus.

FIG. 2. Coronary artery above the caseous focus. (× 100)

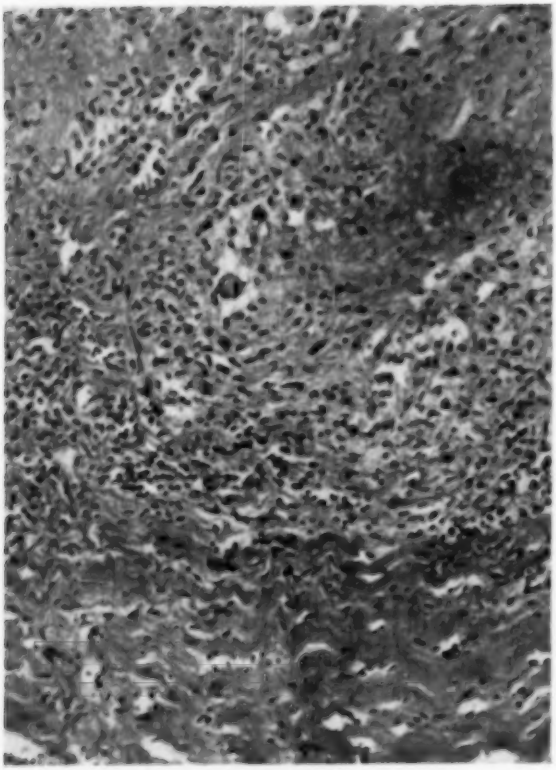

FIG. 3. Tuberculous granuloma with Langhans' giant cell in the center of the field. Mild pressure atrophy of the myocardium below. (× 200)

Microscopic: The predominant lesions were circumscribed areas of caseation necrosis surrounded by epithelioid cells, Langhans' giant cells, and lymphocytes. They were distributed throughout the lungs, liver, spleen, lymph nodes, adrenals, vertebra, brain, prostate, and kidneys. The caseous tuberculous lesions in the heart were adjacent to, but did not involve, the coronary artery (figure 2) and showed the same changes as were observed in the other organs. A moderate amount of pressure atrophy of the surrounding myocardium was present. The endocardium was normal. Stain for acid-fast bacilli in the heart was negative, but was positive in the lung. Severe arteriolar necrosis was present in the kidneys as well as many areas of tubercle formation (figures 3 and 4).

DISCUSSION

The diagnosis of tuberculosis of the vertebra was not made ante mortem because it was believed that the patient had metastatic carcinoma from the thyroid. More attention to the original pathologic report of "tubercle formation" in the thyroid might have established this diagnosis. It appears likely that the radiation therapy to the vertebra had a deleterious effect, and caused rapid dissemination of the tuberculosis. It is of interest that the patient had no cardiac symptoms in spite of the large caseous lesion in the left ventricle.

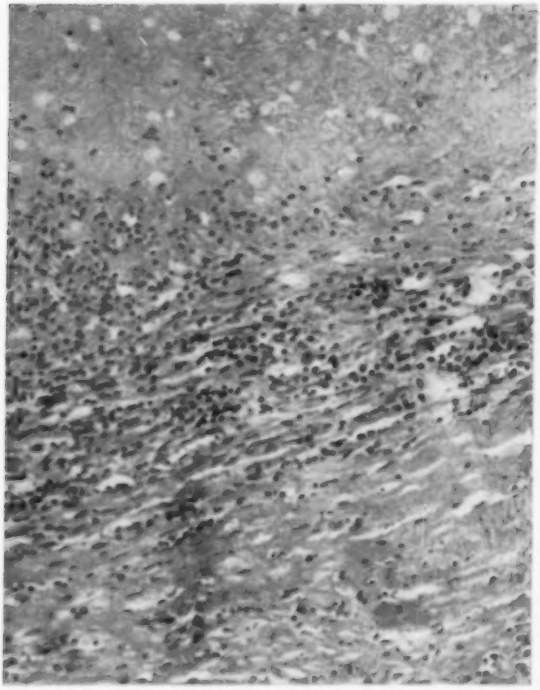

FIG. 4. Granulation tissue in center with caseation necrosis in upper portion of field and myocardium below. (× 200)

Two theories appear repeatedly in the literature to explain the rarity of tuberculosis of the myocardium. The first is that actively contractile tissue is not the optimal medium for the tubercle bacilli to gain foothold and to multiply. Adams, Denny-Brown, and Pearson (6) state that the bacteria are implanted in the connective tissue about the intramuscular fasciculi.

The second theory is that the lactic acid produced in muscle has a detrimental effect on the tubercle bacilus. Dubos (7) has shown in *in vitro* studies that lactic acid prevents the multiplication of tubercle bacilli but does not exert a bactericidal effect. Lactic acid disrupts the metabolism of the organism by increasing its respiration, hence stimulating the oxygen uptake of the tubercle bacillus without cellular multiplication. This causes depletion of reserve substances of the bacteria and accumulation of toxins within the organism. He has found that lactic acid is also produced by autolytic changes in necrotic tissue and has noted that a caseous tuberculous lesion often has a paucity of acid-fast bacilli. Baker (8), in a review at the Johns Hopkins Hospital, did not find a single case of vegetative tuberculous endocarditis at the line of closure of the valves. He quotes Rich to the effect that one reason the tubercle bacillus might not produce tuberculous valvulitis is its relatively slow growth relative to pyogenic bacteria.

Primary tuberculosis of the myocardium probably does not occur. The few cases reported have not been authenticated. Myocardial tuberculosis is secondary to another

focus of the disease; it is involved either by direct extension, lymphatic invasion, or hematogenous spread.

Rich (9) has shown differences in tissue resistance of various organs to tuberculosis and the lack of correlation between the extent of deposition of the tubercle bacilli and the progression of the disease in that organ. The organisms proliferate only in the phagocytes and interstices of organs and obtain nutrition from the phagocytes and the interstitial fluid. The medium differs in the various organs and, since tuberculosis of the myocardium is so rare, one might consider that the tissue fluid of the heart is not condusive to the growth of the tubercle bacillus. He states that the tubercle bacillus within the macrophages must compete with the parenchymatous cells for nutrition which varies from organ to organ. The tubercle bacillus is sensitive to oxygen lack. With decrease in oxygen tension, the growth of the bacillus is markedly inhibited. Rich has shown that progression of tuberculosis in the guinea pig can be retarded by subjecting the animals to an atmosphere low in oxygen. From this and other facts he concluded that the growth and the proliferation of tubercle bacilli in different organs depend on the amount of available oxygen and their competitive ability to remove oxygen for their own metabolism.

SUMMARY

A case of myocardial tuberculosis is presented with a discussion of some of the theories for the reason it is so relatively rare. Tuberculosis of the myocardium, although rare, occurs with sufficient frequency that one must be aware of it in dealing with patients with tuberculosis. The diagnosis is difficult, for many patients with extensive myocardial tuberculosis have no cardiac symptoms.

The diagnosis of tuberculosis of the heart may be suspected in a patient with generalized tuberculosis who suddenly develops cardiac symptoms when previously he had none.

SUMARIO

Tuberculosis del Miocardio

Preséntase un caso de tuberculosis miocardíaca con una discusión de algunas de las teorías que explican su relativa rareza. La tuberculosis del miocardio, aunque rara, alcanza suficiente frecuencia para tener que tomarla en cuenta al atender a tuberculosos. El diagnóstico es difícil, pues muchos casos de extensa tuberculosis miocardíaca no acusan síntomas cardíacos.

Cabe sospechar el diagnóstico de tuberculosis del corazón en un sujeto que tenga tuberculosis generalizada y de repente manifieste síntomas cardíacos sin haberlos tenido antes.

RESUME

Tuberculose du myocarde

Présentation d'un cas de tuberculose du myocarde avec discussion de certaines des théories sur les raisons de sa rareté relative. La tuberculose du myocarde, bien que rare, apparaît avec une fréquence suffisante pour qu'on en tienne compte lorsqu'il s'agit de malades atteintes de tuberculose. Le diagnostic est difficile, car de nombreux cas de tuberculose extensive du myocarde sont dépourvus de signes cardiaques.

Le diagnostic de tuberculose du coeur peut être présumé chez un malade atteint de tuberculose généralisée quand des symptômes cardiaques apparaissent soudainement alors le malade n'en présentait aucun.

Acknowledgment

The writer acknowledges with thanks his indebtedness to Dr. Benjamin S. Gordon, Chief of Laboratory Service, Veterans Administration Hospital, Bronx, New York, for his

aid and cooperation in reviewing microscopic sections; and to the Medical Illustration Service for preparing the photographs.

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CALCIFICATION WITHIN THE SOLITARY PULMONARY NODULE[1]

A Fallible Sign of Benignity

EDGAR W. DAVIS, SOL KATZ, AND J. WINTHROP PEABODY, JR.

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INTRODUCTION

The solitary intrapulmonary nodule, inappropriately termed a "coin lesion" and popularly and far too liberally referred to by the laity as a "spot on the lung," is currently provoking more than routine interest because of its sinister connotation. There is a growing conviction among those who see and follow a good number of patients with such lesions that the malignant lesion cannot be differentiated from the benign one with any degree of consistency by any means short of thoracotomy. Recognizing this fact, the present writers for a long time advocated prompt excision of these lesions and have so expressed themselves on previous occasions (1). The sole exception to this policy has been the nodule containing calcium. The recommendation has been to leave these nodules alone on the ground that they are invariably granulomas or hamartomas, neither of which requires excision if stability can be demonstrated by serial roentgenograms. Although still convinced of the soundness of this attitude, a case has been observed which has shaken confidence in this belief. The patient in this case had a pulmonary nodule which from its apical location, small size, circumscribed margin, and partially calcified center provided every roentgenographic assurance of its benignity, when in truth it was a squamous carcinoma of the lung.

In view of the virtual infallibility attached to the presence of calcium as the sign of a benign lesion, a case of this sort interjects a timely note of caution. This report is intended, not as an argument against the conservative management of the partially calcified pulmonary nodule, but as proof that a fleck or two of calcium does not necessarily impart complete assurance of the benign character of a lesion. Moreover, any increase in size is a cause for alarm regardless of the presence of calcification. The question now arises whether there are any circumstances in which one can temporize with these lesions.

CASE REPORT

J. D., a 40-year-old white woman, began to experience occasional paresthesias of the left arm in August, 1954. Three weeks later moderately severe right occipital headaches appeared and gradually progressed in intensity, until by mid-October they were quite vicious and accompanied by intractable vomiting. Papilledema was then noted. A pneumoencephalogram showed the right lateral ventricle to be pushed to the left by a mass in the right parietal region. On October 29, a right parietal craniotomy was performed by Dr. Jonathan M. Williams, with complete excision of a 3 by 6 cm. cortical tumor.

Pathologic report: Anaplastic squamous carcinoma, metastatic.

Recovery was complete and uneventful. A meticulous search for the primary site revealed no abnormalities other than an unimpressive, partially calcified nodule in the extreme apex of the right lung which the radiologist chose to call a tuberculoma (figure 1). A most scrupulous physical examination revealed no abnormalities nor were there any abnormal findings on multiple laboratory and roentgenographic studies. The cutaneous reaction to tuberculin was positive. Inasmuch as periodic investigations continued to show nothing but the so-called "tuberculoma," the patient was referred to the writers for evalua-

[1] From the Departments of Thoracic Surgery and Medicine, Georgetown University School of Medicine, and Garfield Memorial Hospital, Washington, D. C.

FIG. 1. Chest roentgenogram, obtained on August 25, 1954, interpreted as showing a "healed lesion" in the apex of the right lung. Calcification can be seen within the nodule.

tion of this purportedly benign pulmonary tumor. With her she brought a series of chest roentgenograms which had been previously interpreted as follows:

> 8-25-54. Examination of the chest shows no evidence of abnormality of the bony framework, heart, or great blood vessels. There is some thickening of the pleura and a small amount of infiltration in the right supraclavicular region, a part of which is calcified. This is probably a healed lesion. The lung fields are otherwise clear.
>
> 11-5-54. Re-examination of the chest in comparison with the examination of 8-25-54 shows no change. There is a distinct area of infiltration on the right side in the supraclavicular region which contains some calcium and probably represents an old tuberculous lesion. The lung fields are otherwise clear. There may be a faint possibility that this could represent new growth, but that seems very unlikely as it is continuous with the pleura and has a rather characteristic appearance of an old tuberculous lesion.

Review of these roentgenograms confirmed the impression of calcium within the right apical nodule but, of greater importance, these films suggested a slight but perceptible increment in the size of the lesion (figure 2). The latter finding, coupled with an undiscovered primary site and otherwise negative clinical examinations, lent strong support to the possibility that this might represent a bronchogenic carcinoma.

In view of the patient's emotional stability and realistic attitude, this opinion was frankly presented to her. Although acquainted with the improbability that excision would alter the prognosis, she was adamant in wanting the nodule excised.

On January 13, 1955, under cyclopropane-oxygen-ether endotracheal anesthesia, the right pleural space was entered through the bed of the subperiosteally excised fourth rib. The apex of the right upper lobe presented a number of thin but highly vascular adhesions.

FIG. 2. Chest roentgenogram, obtained on November 11, 1954, interpreted as having "a rather characteristic appearance of an old tuberculous lesion." There has been a definite increase in size since the previous roentgenogram.

After freeing the lung, two firm nodules could be palpated in the extreme apex in close proximity to each other. One involved the visceral pleura. In the absence of palpable mediastinal lymph nodes and presence of known distant metastasis, a wedge resection was performed. The severed edge of the lung was closed by a double-tiered, continuous chromic catgut suture on an atraumatic needle. Hemostasis and pneumostasis were complete. A water-seal drain was established and the chest was closed in routine fashion. The postoperative course was devoid of complications. In the period since operation the patient has remained well.

A roentgenogram of the excised wedge of lung showed several small flecks of calcium (figure 3). Grossly there was a granular pleural plaque approximately one centimeter in diameter. On cut section two white nodules were seen (figure 4). Microscopic examination showed both nodules to be comprised of squamous carcinoma resembling that removed from the brain. The nodule invading visceral pleura presented many small calcific deposits. Histologically it was impossible to tell whether the tumor had produced or coincidentally invaded the areas of calcification.

The pathologic diagnosis was bronchogenic carcinoma of the squamous cell type with pleural invasion.

DISCUSSION

It is generally conceded that calcification within a solitary pulmonary nodule practically guarantees its benign character. In an extensive roentgenographic experience at the Mayo

FIG. 3. Roentgenogram of the resected specimen showing a nidus of calcium. Microscopic examination confirmed the presence of calcium within the tumor.

FIG. 4. Photograph of the transected specimen. Note the two juxtaposed nodules, both comprised of squamous carcinoma, the more superficial one containing calcium.

Clinic, Good claims never to have observed calcium in a primary malignant pulmonary nodule (2). However, in the discussion following his paper, Hodes mentioned four patients with calcified nodules which ultimately revealed bronchogenic carcinoma. Casual reference has twice been made to a bronchogenic carcinoma which roentgenographically appeared to contain calcium (3). Two similar cases have been reported in detail (4) and microscopic evidence of calcification has been encountered in still another (5). Whether through theory or sad experience, in most recent publications on this subject the writers have been reluctant to place complete dependence upon the presence of calcium as a guide to the character of a lesion, and either deny or at least question the reliability of this sign.

The present writers are not so willing to ignore the significance of calcification within a lesion, however, and remain firmly convinced that the finding has value in avoiding much unnecessary surgery. Needless to say, one must be sure that calcium lies within the tumor and that it is not simply one of many calcific deposits in other areas of the lung. Of paramount importance is the type of calcification. Lesions which are diffusely stippled with calcium, lesions presenting a calcific outer ring or inner laminations, and lesions containing a relatively large central core of calcium are almost assuredly granulomas and, if stable, should be left alone. Conversely, the presence within a pulmonary nodule of a few tiny bits of calcium cannot be construed as sound evidence of a benign lesion. From experience with the present case it may be seen that the solitary pulmonary tumor containing minimal calcification is a potentially dangerous lesion. Like those without calcification it can be more safely excised than ignored.

SUMMARY

The presence of calcification within a solitary intrapulmonary tumor is commonly regarded as evidence of its benign character. Excision is ordinarily considered unnecessary.

A case is reported in which a small circumscribed, partially calcified, innocuous-looking nodule in the apex of the right upper lobe proved to be a bronchogenic carcinoma. That the calcification lay within the tumor was confirmed by microscopic examination.

Whereas calcification in the form of diffuse stippling, inner laminations, an outer ring, or a relatively large central core almost assuredly indicates a granuloma, a few flecks of calcium are much less reassuring.

When there exists a disproportion between the size of the nodule and the degree of calcification, it may be safer to excise rather than observe the lesion. Certainly any increase in the size of the nodule is a cause for alarm regardless of the presence of calcification.

SUMARIO

La Calcificación Dentro del Nódulo Pulmonar Solitario Signo Falible de Benignidad

La presencia de calcificación dentro de un tumor intrapulmonar solitario está considerada comúnmente como signo de benignidad, considerándose ordinariamente entonces innecesaria la excisión.

Preséntase un caso en el que un nódulo pequeño, circunscrito, parcialmente calcificado, de aspecto inocuo en el vértice del lóbulo superior derecho resultó ser carcinoma broncógeno. El examen microscópico confirmó que la calcificación quedaba dentro del tumor.

En tanto que la calcificatión en forma de granulación difusa, láminas internas, anillo externo, o de un núcleo relativamente grande, indica casi seguramente granuloma, hay que convenir en que algunos copillos de calcio son mucho menos alentadores.

Cuando existe desproporción entre el tamaño del nódulo y el grado de calcificación, tal ves sea más prudente excindir que no seguir observando la lesión. Ciertamente, todo aumento del tamaño del nódulo es motivo de alarma independientemente de la presencia de calcificación.

RESUME

Calcification à l'intérieur du nodule pulmonaire solitaire: signe faillible de bénignité

La présence de calcification à l'intérieur du nodule solitaire intrapulmonaire est habituellement considérée à tort comme une preuve de bénignité. L'excision est ordinairement considérée inutile.

Les auteurs rapportent un cas dans lequel un petit nodule circonscrit, partiellement calcifié, d'aspect bénin inoffensif, situé à l'apex du lobe supérieur droit fut identifié comme étant un cancer bronchogénique. L'examen histologique a confirmé que la calcification était située à l'intérieur de la tumeur.

Tandis que la calcification sous la forme de pointillé diffus, de lamelles à la partie interne, d'un anneau externe ou d'un noyau central relativement volumineux indique presque sûrement le granulome. Il y a lieu de conclure que la présence de quelques rares particules de calcium est beaucoup moins rassurante.

Quand il existe une disproportion entre le volume du nodule et le degré de calcification il est plus sûr d'exciser la lésion que de l'observer. Il est certain que toute augmentation de volume du nodule est une cause d'alarme, avec ou sans présence de calcification.

Addendum

Since the writers submitted this article for publication, the patient discussed in the case report returned to the hospital with recurrent signs of cerebral metastasis. She died in February, 1956. At autopsy, the only residual evidence of carcinoma was in the right temporal lobe of the brain.

Prompted by the findings in this case, the writers sent questionnaires to all members of the American Association for Thoracic Surgery to determine whether the occurrence of carcinoma in the partially calcified solitary pulmonary nodule is as rare as is generally believed. Surprisingly enough, 114 members claimed to have encountered a malignant nodule containing calcium, and 76 of these men described 155 such cases in detail. In practically all instances, the calcification consisted of a few flecks, although isolated examples of moderately and extensively calcified solitary nodules were also cited. A small number with slight central calcification was mentioned, as well as one with a calcified capsule, but there was none with a large central core, an inner ring, or concentric laminations of calcium. There was general agreement that minimal calcification is no guarantee against malignancy. This survey will be the subject of a subsequent report.

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THE PRESENCE OF LUPUS ERYTHEMATOSUS CELLS IN MILIARY TUBERCULOSIS[1]

HARRY W. HAVERLAND

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INTRODUCTION

Since the discovery of the lupus erythematosus (L. E.) cells in the disease of that name, false positive reactions have been relatively few (1, 2). In only two instances, so far as could be ascertained from the available literature, has the L. E. phenomenon been reported in cases of miliary tuberculosis (3, 4). The case reported by Jacobs (4) bears a striking similarity to the present case, in which detection of L. E. cells led to a clinical diagnosis of disseminated lupus erythematosus.

CASE REPORT

The patient was a 64-year-old woman who was last admitted to the Millard Fillmore Hospital on March 23, 1955, and died two days later. She had had two previous admissions, the first being in January, 1953, at which time she complained of anorexia and weight loss of six months' duration. There was no history of tuberculosis. Physical examination at that time showed the patient to be poorly nourished and to have a brownish pigmentation of the legs. The mucous membranes were clear; examination showed that the chest was normal; the heart was not enlarged. The blood pressure was 70 systolic and 50 diastolic in mm. of mercury. No abdominal organs were palpably enlarged. There was no lymphadenopathy. Her temperature on admission was 101°F., and ranged from 100° to 102° F. during her hospital stay.

The hemoglobin content of the blood at the time of this first admission was 9.4 gm. per 100 ml. The erythrocytes numbered 3,340,000 and the leukocytes 4,500 per cu. mm. of blood, with a differential count of 73 per cent filaments, 11 per cent band forms, 8 per cent intermediate lymphocytes, 6 per cent small lymphocytes, and 2 per cent plasma cells. There was slight to moderate anisocytosis. There were 205,000 blood platelets per cu. mm. and the reticulocyte count was 0.4 per cent.

The urine specific gravity was 1.014 and there was 2-plus albumin. The result of the cephalin flocculation test was 4-plus in twenty-four hours; and that of thymol turbidity, 18 units. The erythrocyte sedimentation rate (Wintrobe method) uncorrected was 62 mm. per hour; corrected, 31 mm. per hour.

The blood urea nitrogen was 20 mg. per 100 ml.; total serum protein, 7.2 gm.; and serum albumin, 3.56 gm. per 100 ml. Serologic tests for syphilis yielded negative results.

Bone marrow examination showed essentially normal activity with slight myeloid preponderance and slight left shift to late myelocyte and band forms. A few plasma cells and histiocytes were seen. Cystoscopic examination yielded negative findings. Cultures and smears of bone marrow showed no organisms. The patient was given 1 gm. of streptomycin daily from January 9 to 22, 1953.

Her second admission was on December 31, 1954, for fever of undetermined origin which ranged from 99° to 102° F. rectally and was accompanied by extreme weakness. On examination, again the chest was normal, and the heart was not enlarged. The blood pressure was 132 mm. systolic and 64 diastolic in mm. of mercury; the pulse rate, 92 beats per minute; the heart rhythm, regular. There was a soft grade I systolic aortic murmur. The abdomen was soft and no masses or organs were palpated. Roentgenographic examination of the chest disclosed no abnormalities.

[1] From the Department of Pathology, Millard Fillmore Hospital, Buffalo, New York.

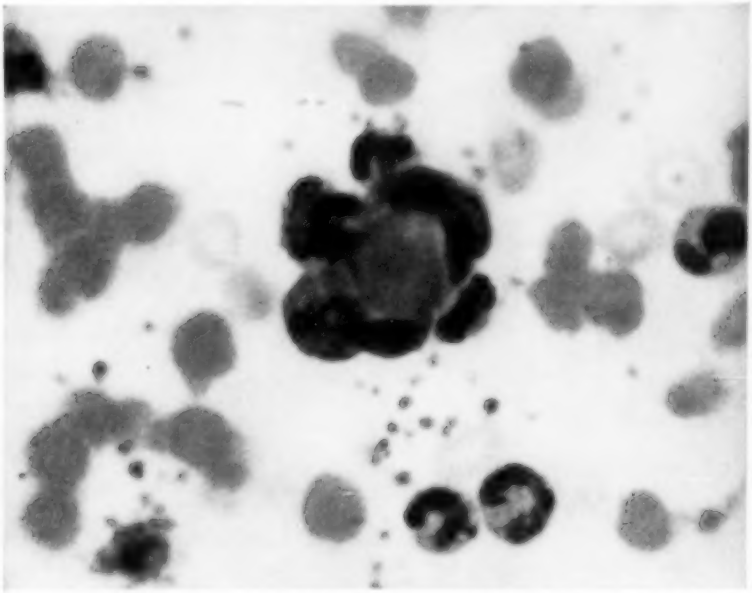

FIG. 1. The characteristic rosette as well as neutrophils are shown in this photomicrograph.

The hemoglobin content of the blood was 9.15 gm.; the erythrocytes numbered 3,500,000 and the leukocytes 3,000 per cu. mm. of blood, with a differential of 77 per cent filaments, 13 per cent band forms, 1 per cent intermediate lymphocytes, and 9 per cent small lymphocytes. The specific gravity of the urine was 1.014; the albumin was 2-plus, and an occasional erythrocyte and from 7 to 10 leukocytes per high-power field as well as many bacteria were seen.

The total serum protein was 7.07 gm. and the serum globulin 3.61 gm. per 100 ml. The serum albumin was 3.46 gm. per 100 ml. Blood cultures disclosed no abnormalities. A sternal bone marrow examination on January 5 showed normal myeloid activity. Erythroid elements were somewhat depressed, but no maturation defect was noted. There was a slight increase in plasma cells.

An L. E. preparation done on venous blood on January 7 was definitely positive. There were forms interpreted as rosettes (figure 1) together with moderate numbers of L. E. cells containing homogenous pink-staining material (figure 2). Some nucleophagocytosis was also present. There was approximately one L. E. cell or group of cells per low-power field of a buffy coat preparation of heparinized blood. Platelet clumps were frequent. The diagnosis of disseminated lupus erythematosus was made.

The patient's rectal temperature, which had ranged from 100° to 103° F., receded toward normal, beginning on January 10 and was normal on discharge on January 15, 1955. Penicillin, 400,000 units, and streptomycin, 0.5 gm., were administered daily from January 3 to the day of discharge. Cortisone, 20 mg. four times daily, was given concomitantly, beginning on January 11 and was continued after the patient's discharge in decreasing weekly dosage, being maintained at 20 mg. per day. There was no evidence of untoward reaction to the drugs at any time, and the patient remained afebrile until a few days before her final admission.

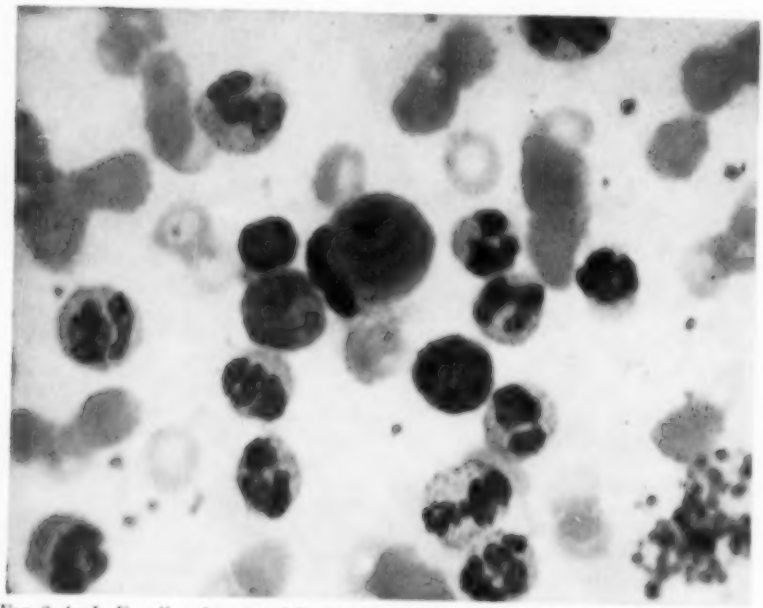

FIG. 2. An L. E. cell and neutrophils are shown. The preparation was done on a buffy coat of heparinized venous blood.

The patient was in a terminal state when she was admitted on March 23, 1955. Her temperature was 102° F.; there were occasional rales at the base of the right lung; the heart rate was rapid, but no murmurs were noted; and the blood pressure was 100 mm. systolic and 70 diastolic in mm. of mercury. The abdomen was normal on palpation.

The hemoglobin content of the blood was 10.7 gm. There were 3,570,000 erythrocytes and 1,850 leukocytes per cu. mm. of blood, with a differential count of 17 per cent filaments, 80 per cent band forms, 1 per cent intermediate lymphocytes, and 2 per cent small lymphocytes. Coarse toxic granules were noted in the neutrophils. The patient died on March 25.

Post-Mortem Findings

Necropsy showed cachexia. No lymphadenopathy was noted. The pleural cavities were obliterated by easily lysed, fibrous adhesions. The heart weighed 390 gm. Pronounced arteriosclerosis of the coronary arteries was found.

Both lungs were similar in appearance, together weighing 1,410 gm., grayish-black in color, voluminous and nodular, some nodules measuring up to 1 cm. in diameter. On cut section they showed moist surfaces studded with clover-leaf, raised, grayish-white, soft nodules. Tracheobronchial and bronchopulmonary nodes averaged 1.5 cm. in diameter and were black and rubbery. Microscopic sections revealed miliary tubercles, some exhibiting organization. Many others were more recent and had nodular confluent areas of acute necrosis with remarkable absence of cellular response. Some nodules contained caseation and giant cells. Changes consistent with tuberculous pneumonia were seen in some areas and in others, between tuberculous foci, there was extensive edema.

The liver weighed 1,120 gm. Grossly, scattered yellowish nodules, measuring up to 2 cm., were seen. Microscopically, numerous miliary tubercles of various sizes were seen, some showing acute necrosis and most containing Langhans' giant cells. The tubercles were all located within the portal areas and were surrounded by a slight lymphocytic infiltrate.

The spleen weighed 200 gm.; its capsule was rough and diaphragmatic adhesions were noted. Several nodules, measuring up to 0.6 cm., were scattered throughout the parenchyma. Microscopically the lesions varied greatly in size, some of the larger ones being confluent, multiple small tubercles. One of the larger lesions showed peripheral fibrosis.

The gastrointestinal tract was not remarkable except for a transverse, shallow mucosal ulcer, 1 cm. in diameter, lying 15 cm. distal to the ileocecal junction. The ulcer showed acute fibrinoid necrosis but no giant-cell reaction.

The kidneys each weighed 170 gm. Several 0.2 cm. yellow nodules noted on microscopic section proved to be areas of necrotic tubercle formation surrounded by giant cells and lymphocytes. The adrenals, uterus and ovaries, tubes and pancreas were not remarkable. Sections of periaortic, mediastinal, and bronchopulmonary nodes revealed healing tuberculosis with prominent fibrosis in addition to active foci of necrosis. The only pathologic changes found in the brain, grossly and histologically, were generalized edema and hyperemia in the subependyma.

Multiple sections of bone marrow disclosed hypoplasia with fatty infiltration and areas of focal necrosis similar to that seen in other areas except for a lack of giant cells. There was a predominance of myelocytes with occasional plasma cells and a definite erythroid depression. Acid-fast bacilli were not demonstrated in bone marrow. The lungs, however, contained numerous acid-fast bacilli in the larger necrotic areas, and many such bacilli were also seen in the liver, spleen, and kidneys.

Cultures of lung tissue grew large numbers of acid-fast microorganisms resembling *Mycobacterium tuberculosis* by the end of two weeks.

In summary, there was post-mortem evidence of widespread miliary tuberculosis of the lungs, liver, spleen, kidneys, and lymph nodes, as well as the bone marrow, but there was no primary focus in the lungs. A tuberculous pneumonia had destroyed large areas of the pulmonary parenchyma. Acid-fast stains of the lungs, spleen, and kidneys disclosed numerous acid-fast microorganisms. Cultures of lung tissue were positive in two weeks for organisms resembling *M. tuberculosis*. There were no changes in any organ which could be attributed to lupus erythematosus.

COMMENT

The diagnosis of miliary tuberculosis ante mortem has always been difficult. This difficulty is increased if the L. E. cell phenomenon proves to be a common or even only an occasional finding associated with miliary tuberculosis.

Recently cases have been reported in which the L. E. cell phenomenon occurred in patients with severe reactions to penicillin (1) as well as to hydralazine therapy (2, 5, 6). Both the patient in the case reported here and Jacobs' patient (4) received penicillin, but neither patient experienced the severe reaction which appears necessary to produce the L. E. phenomenon. The former patient also received streptomycin on the two first admissions, but a streptomycin-induced L. E. phenomenon has never been suggested.

There was a persistent reversal of the albumin-globulin ratio in the present case. This is often noted in lupus erythematosus and may be seen in tuberculosis. One might speculate concerning the relationship between the plasma factor involved in the L.E. phenomenon (5) and the alteration in plasma proteins produced by the tuberculous process, making it possible for tuberculosis to produce an L. E. phenomenon without clinical demonstration of lupus erythematosus.

SUMMARY

A case has been presented of a patient who died following a chronic, febrile, wasting illness. Laboratory tests showed a persistent anemia, leukopenia, and relative neutrophilia. Miliary tuberculosis was found at necropsy. Systemic lupus erythematosus was sus-

pected clinically, and L. E. cells were demonstrated on one occasion, but no organic changes suggestive of lupus erythematosus were found at necropsy.

SUMARIO

El Lupus Eritematoso en la Granulia

Preséntase el caso de un sujeto que falleció consecutivamente a una enfermedad extenuante, febril, crónica. Las pruebas de laboratorio revelaron anemia persistente, leucopenia y neutrofilia relativa. En la autopsia se descubrió granulia. Clínicamente, se sospechó lupus eritematoso generalizado y en una ocasión se descubrieron células del mismo, pero en la autopsia no se encontraron alteraciones orgánicas indicativas de esta dolencia.

RESUME

Le lupus érythémateux dans la tuberculose miliaire

Observation d'un malade qui a succombé à une maladie chronique, fébrile et cachectisante. Les tests de laboratoires montraient une anémie persistante avec leucopénie et neutrophilie relative. La tuberculose miliaire fut découverte à l'autopsie. On soupçonnait cliniquement le diagnostic de lupus érythémateux disséminé et des cellules L. E. ont été découvertes à l'occasion d'un examen, mais aucune lésion organique suggérant cette affection n'a été découverte à l'autopsie.

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Editorial

Chemoprophylaxis, Immunity, and Prevention in Tuberculosis

There have been published recently a number of experimental studies and semi-official statements dealing with the use of isoniazid "as a general preventive of tuberculosis."[1] Explicitly or implicitly, it is claimed in these publications that isoniazid chemoprophylaxis can prevent tuberculosis and yet allow antituberculous immunity to develop in exposed persons.

Two statements may be quoted to illustrate the views of those who hold that chemoprophylaxis can be regarded as equivalent to vaccination. In a paper on "Prevention of Experimental Tuberculosis with Isoniazid," recently published in the REVIEW,[2] the conclusion is reached that "the degree of resistance observed" in animals infected while under chemoprophylaxis "appears to be comparable with that produced by vaccinating guinea pigs with BCG." In a reference to this same study appearing in the annual report of the National Tuberculosis Association, it is stated even more forcefully that: "using isoniazid as a *preventive* measure (italics mine) . . . so far results appear better than those obtained with BCG."[3]

The belief that chemoprophylaxis can be equated with both prevention and vaccination is being used as the justification for large-scale trials in man. There is some urgency, therefore, in examining the scientific basis and practical implications of these trials.

On superficial examination, the claim that immunity to tuberculosis can develop in persons exposed to bacilli while under the cover of isoniazid appears to be supported by recent experimental studies in mice and in guinea pigs. More careful scrutiny, however, shows that the design and execution of these experiments do not justify the inferences that have been derived from their results. Since lack of space would not permit here an adequate discussion of all of the relevant technical details, it will suffice to consider briefly only some limited aspects of the design of two studies published in the REVIEW.[2, 4]

The first of these papers deals with vaccination of mice with BCG vaccine.[4] Its conclusion is that "A high dose of isoniazid given throughout the pre-challenge vaccination period did not significantly reduce the immunizing properties of BCG vaccine. This indicated that the bacterial multiplication of a vaccine is not a factor essential for the degree of immunity obtained." This conclusion is derived from the results of experiments in which mice "were vaccinated intraperitoneally on different days with 0.1 ml. of five-day Tween®-albumin cultures of BCG"—a truly formidable dose.

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As pointed out by the writers themselves, the view that "bacterial multiplication is not an essential factor in this type of vaccination . . . is based on the assumption that isoniazid inhibits multiplication of BCG in the mouse." In my opinion, there is no ground for regarding this assumption as valid, in particular when one considers the conditions under which the tests were performed. For it is most unlikely, indeed contrary to available evidence, that isoniazid can immediately and completely prevent the multiplication *in vivo* of a large dose of young BCG cells, especially when these are introduced into the peritoneal cavity, as was the case in this study. It is obvious, furthermore, that even a limited degree of proliferation of the large BCG inoculum used (0.1 ml. of culture!) would have resulted in a marked increase of the bacillary mass *in vivo*. Thus, the very design of these experiments made it impossible to derive from their results conclusions concerning the influence of chemoprophylaxis on the effectiveness of BCG vaccine. In order to obtain information relevant to the effect of isoniazid on vaccination as normally practiced, it would have been necessary to use a much smaller dose of BCG, and to administer it by some route other than intraperitoneal.

Similar defects in design can be recognized in the second study mentioned above.[2] In this study, the authors injected large amounts of a culture of H37Rv into guinea pigs which they treated with isoniazid before, during, and after infection. They found that the treated animals resisted the infection, not only during administration of the drug, but also for weeks after therapy had been discontinued. Furthermore, the treated animals exhibited increased resistance to infection when again challenged with a large infective dose.

It must be noted that these findings correspond to experiments in which the infective inoculum was "0.5 ml. of a liquid Tween-albumin suspension of H37Rv" injected by the intraperitoneal route; "the challenge contained approximately 5×10^6 viable units per ml." On the basis of long experience with Tween-albumin cultures of tubercle bacilli and with the limitations of the colonial enumeration techniques, I feel confident that the challenge inoculum used in these experiments contained approximately 10^9 bacterial cells per ml. (determined by microscopic counts). As mentioned earlier in the case of mice infected with BCG, there is much reason to believe that injection of such an enormous dose by the *intraperitoneal route* resulted in significant bacterial multiplication *in vivo*, even under the cover of isoniazid. In consequence, the guinea pigs under consideration had been subjected to an intense antigenic stimulus from the very beginning of the experiment—whether or not they had received chemotherapy. The challenge dose corresponded in other words to an established disease and provided enough bacillary material to elicit immunity. Thus, in this case again, the very design of the experiments made it impossible to derive information concerning the effect of prophylaxis on the development of immunity under natural conditions of exposure.

As is well known, an arrested primary infection does confer some immunity to reinfection and, accordingly, the treated guinea pigs infected with huge bacillary doses naturally proved more resistant to a second challenge dose than did noninfected controls. It is worthy of notice in passing that animals treated with

5 mg. of the drug exhibited "a greater degree of host resistance" to reinfection than did those treated with 25 mg.—a result probably due to the fact that the lower dose had permitted more extensive bacterial proliferation and greater accumulation of antigenic material in the tissues.

It seems hardly necessary to point out that the conditions of these experiments bear little relation to tuberculosis produced by air-borne infection. Since only particles 1 to 5 micra in size can reach the pulmonary tissue, the infective dose in man is relatively small even under conditions of heavy exposure. In a person receiving chemoprophylaxis, the few bacilli thus introduced would undergo only limited multiplication, and could hardly elicit a significant protective immune reaction. Despite a few statements to the contrary which have recently appeared in the literature and which are so undocumented as not to be worth discussing here, it is certain that immunity can develop only when an adequate amount of antigen is present in the tissues—either introduced as a vaccine or produced by extensive multiplication of the infective agent *in vivo*.

As already mentioned, much emphasis has been based on the fact that the administration of isoniazid to heavily infected animals results in a degree of acquired resistance "comparable with that produced by vaccinating guinea pigs with BCG."[2] It is worth keeping in mind the potential danger of an immunity thus obtained. Would space permit, it could be shown that the experiments quoted were totally unsuited to justify the statement that the effect of the drug was "more than a temporary holding action," for it is well known that virulent bacilli usually persist in the tissues even after prolonged chemotherapy. Indeed, it can be assumed that thousands of organisms had survived the course of therapy and that they would have eventually produced progressive disease, had the experiment been continued for a sufficient length of time. How long it would take for reactivation of the infectious process to take place cannot be foretold from available information. To think constructively on the subject would demand knowledge of the effect of the size of the infective inoculum, and of the age and physiologic state of the guinea pigs, on the rate of development of disease. In the absence of this essential information it seems unwarranted to state that ten weeks' therapy was "sufficient to *prevent* (italics mine) the appearance of disease after the drug was discontinued." The paper under consideration does not deal with *prevention* of tuberculosis, but rather with *arrest* of disease.

It seems to me in conclusion that the findings just discussed merely confirm once more two well-established facts: (*a*) tuberculosis caused by a drug-susceptible organism is unlikely to progress under isoniazid therapy; (*b*) a primary infection confers a certain degree of resistance to reinfection, but not necessarily to reactivation of a latent process. This seems a rather slim basis on which to advocate a promiscuous distribution of isoniazid to human beings. And yet it has been published that the U.S.P.H. Service is planning a large-scale trial involving "at least 100,000 persons" in a Latin American country,[5] with the hope that isoniazid prophylaxis will achieve both prevention and vaccination.

As one reads the semi-official statements released to the press, and even some

[5] Mass clinical trial to assay isoniazid as a TB preventive, Medical News, January 16, 1956, *2*, pp. 1 and 6.

of the more technical papers, it is obvious that the words "chemoprophylaxis" and "prevention" are used loosely with several different meanings. To *prevent* the establishment of infection is of course the ideal of chemoprophylaxis; it is not the same as to prevent (or minimize) the chances that primary infection will evolve into active disease; and this in turn is vastly different from decreasing the spread of bacilli by adequate therapy of tuberculous patients. Explicit differentiation among these potential uses of isoniazid would facilitate the design of meaningful experiments in animals or men, and the interpretation of their results. It might lead also to a more precise definition of the possible use of isoniazid in the treatment of primary infection, and even for true prophylaxis. Obviously, there are many problems that cry out for accurate formulation and for pointed experimentation.

Some of the problems will not be readily solved. One need only mention the dangers of drug resistance; the practical difficulties in enforcing continuous administration of the drug for a long enough time; the toxic manifestations of isoniazid (which, although rare, will become important on a population scale); and the many other pitfalls of chemotherapy carried out without medical supervision.

Other problems, fortunately, lend themselves more readily to unambiguous formulation and to laboratory and clinical tests properly designed.

Can chemoprophylaxis really prevent the establishment of infection? And for how long?

Will immunity develop as a result of mere exposure to small numbers of tubercle bacilli, and without extensive bacillary multiplication?

Can prolonged therapy of asymptomic primary infection eventually bring about destruction of all of the bacilli? And will immunity persist after the infection has been truly eradicated or even brought down to a very low level?

Does the decrease in the dangerous consequences of primary infection that will be achieved by isoniazid therapy compensate for the incidence of toxic reaction that may well result from widespread use of the drug? How do the different age groups differ in this respect?

These are but a few of the questions that must be asked and that can be answered by experimentation in animals or by careful observations in man.

In the absence of relevant information, it seems to me of questionable wisdom to advertise the hope that administration of isoniazid constitutes a short cut and safe approach to immunity. It is pointless to state that "whole populations could be treated for as little as a penny a day per person"[1-5] since, in reality, there is no evidence that true prophylaxis can be achieved for long periods of time and no knowledge concerning the bad consequences that may result from the uncontrolled use of isoniazid. *Primo non nocere*. In my opinion, it is a dangerous and backward step to speak of the routine administration of a drug as a "preventive" measure. The word "prevention" has long had a precise meaning in tuberculosis control. It symbolizes a great and exacting tradition that has proved its worth.

René J. Dubos
New York, New York

Notes

CYCLOSERINE THERAPY IN TUBERCULOSIS IN HUMANS

A new antibiotic substance, cycloserine,[1] isolated by Harned and associates[2] from cultures of *Streptomyces orchidaceous*, was found to have significant *in vitro* activity against tubercle bacilli by Welch and co-workers[3] and was first studied in man by Epstein and associates.[4] These investigators described the results obtained with the administration of cycloserine to 37 patients with pulmonary tuberculosis. They found that 36 patients who were treated with cycloserine showed clinical improvement, weight gain, reduced fever, and that 30 of them demonstrated sputum "conversion" as determined by microscopy. In 28 of these patients, simultaneous improvement of chest roentgenograms occurred. These results were obtained on dosages of 1.0 to 1.5 gm. of cycloserine per day over a period of six to sixteen weeks.

The present report summarizes the results obtained in this laboratory with cycloserine therapy in 10 male patients, each of whom was treated for at least four months.

All of the patients had been observed for a sufficient time prior to the start of cycloserine therapy to determine the trend of their disease (table 1). In 9 patients the disease was far advanced, and in one it was moderately advanced. All of the 10 patients had chronic disease with roentgenographic evidence of recent progression. Eight patients had multiple cavitation, one a single cavity, and one patient had generalized involvement of both lungs due to silicotuberculosis. A left upper lobectomy had been performed for residual cavitary disease in the last patient. One other patient had received surgical collapse therapy by means of a left extraperiosteal thoracoplasty with Lucite® ball plombage. In both instances, the surgical procedures had been performed several years before the patients were admitted to the present study.

Eight of the 10 patients had had known pulmonary tuberculosis for several years before inclusion in the present study and they had received antimicrobial therapy with streptomycin, isoniazid, and para-aminosalicylic acid (PAS) in various combinations for varying lengths of time. All of these 8 patients had failed to respond to such therapy, and the degree of resistance of tubercle bacilli in their sputum prior to the initiation of treatment with cycloserine is summarized in table 2. The status of sputum cultures before and after the patients received cycloserine therapy is shown in table 3. It will be noted that all patients included in the study group had sputum positive for *M. tuberculosis* on both microscopy and culture before cycloserine therapy was initiated.

The study was designed to yield information only with respect to the general efficacy of cycloserine therapy and its clinical toxicity at moderate dosage. Therefore, no other antituberculous drugs were given to the selected patients during its administration. After careful physical examination and clinical evaluation, the patients were selected on the basis of failure to respond satisfactorily to conventional chemotherapy, and the expectation that these patients would remain in the hospital for at least six months. The 2 patients who had received no prior chemotherapy were included in the series in an effort to gain

[1] Kindly supplied for this study, as Seromycin®, by Dr. R. S. Griffith, Eli Lilly and Company, Indianapolis 6, Indiana.

[2] HARNED, R. L., *et al.*: Antibiot. Med., 1955, *1*, 204.

[3] WELCH, H., *et al.*: Antibiot. Med., 1955, *1*, 79.

[4] EPSTEIN, I. G., *et al.*: Antibiot. Med., 1955, *1*, 80.

TABLE 1

CLINICAL RESPONSE TO CYCLOSERINE THERAPY

Case Number Initial Record Number	Age	Race	Diagnosis	Date of Diagnosis of TB	Previous Chemotherapy	Cycloserine Duration of Therapy	Cough and Expectoration	Weight Gained	Apparent Clinical Response
						days		*pounds*	
1 E.L. A-1079	29	N	Far advanced pulmonary TB, bilateral with multiple cavitation	11/1/49	X	220	Decreased	26½	Very good
2 T.M. A-2587	45	W	Far advanced pulmonary TB, bilateral multiple cavitation, L. extraperiosteal plombage	6/27/50	X	227	Decreased	6	Good
3 R.S. A-54892	41	W	Far advanced pulmonary TB, bilateral with multiple cavitation	10/1/53	X	225	Decreased	14	Good
4 J.McB. A-2796	51	W	Far advanced pulmonary TB, bilateral with multiple cavitation	8/1/53	X	227	Decreased	5	Good
5 F.O. A-56030	44	W	Far advanced pulmonary TB, bilateral with multiple cavitation	6/10/53	0	198	Decreased	17	Good
6 W.K. A-9178	62	W	Far advanced pulmonary TB, bilateral, left upper lobectomy, 1953	5/10/51	X	160	None	14½	Very good
7 G.O. A-54669	28	W	Moderately advanced pulmonary TB with cavitation	4/7/55	0	236	None	0	Very good
8 L.V. A-3099	47	W	Far advanced pulmonary TB, bilateral with multiple cavitation	9/25/49	X	132	Decreased	10	Good
9 C.B. A-11053	69	W	Far advanced pulmonary TB, bilateral with multiple cavitation	9/27/54	X	139	Unchanged	2½	Good
10 J.S. A-51344	44	W	Far advanced pulmonary TB, bilateral with cavitation	3/15/45	X	190	Unchanged	0	Very good

TABLE 2

DEGREE OF DRUG-RESISTANCE OBSERVED IN CULTURES OF TUBERCLE BACILLI OBTAINED IMMEDIATELY BEFORE THE INITIATION OF CYCLOSERINE THERAPY

Case Number	Highest Concentration in γ per Ml. of Medium in Which Growth Equal to That of Control Was Seen		
	Streptomycin	Isoniazid	PAS
1	3	5.0	0.1
2	10	5.0	10.0
3	3	<0.2	1.0
4	3	<0.2	10.0
5	<3	<0.2	0.1
6	100	5.0	10.0
7	<3	<0.2	0.1
8	3	5.0	0.1
9	100	5.0	0.1
10	100	5.0	1.0

TABLE 3

SUMMARY OF SPUTUM STUDIES BY MICROSCOPY AND CULTURE BEFORE AND AFTER CYCLOSERINE THERAPY

Case Number	Before Therapy		After Therapy	
	Microscopy	Culture	Microscopy	Culture
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	−	+
5	+	+	−	+
6	+	+	+	+
7	+	+	−	−*
8	+	+	+	+
9	+	+	+	+
10	+	+	+	+
Total	10/10	10/10	7/10	9/10

* Specimen removed by segmental resection was positive for acid-fast bacilli on microscopy and negative on culture.

information concerning the response of such patients when initially treated with cycloserine.

Complete blood counts and urinalyses were done initially and repeated every two weeks on all patients; blood urea nitrogen, phenolsulfonphthalein and bromsulphthalein tests, and cephalin flocculation determinations were made bimonthly or more often when indicated. A daily chart of temperature and pulse was maintained, and physical findings and body weight were recorded weekly.

In order to assess the progress of the disease and the response to cycloserine therapy, roentgenograms of the chest were obtained and sputum examinations were made immediately before commencing treatment and were repeated every two weeks thereafter.

TABLE 4

LEVEL OF RESISTANCE TO CYCLOSERINE IN MICROGRAMS PER MILLILITER DURING COURSE OF THERAPY*

Case Number	Pretreatment	After Initiation of Therapy		
		7 Days	30 Days	Final
1	1.25	1.25	5.0	10.0
2	5.00	5.00	10.0	20.0
3	1.25	2.50	5.0	5.0
4	1.25	5.00	5.0	10.0
5	1.25	2.50	2.5	5.0
6	—	10.00	—	20.0
7	5.00	—	—	—
8	2.50	—	5.0	5.0
9	2.50	—	5.0	5.0
10	—	—	—	10.0

* Defined as that concentration of cycloserine in γ per ml. at which growth equivalent to that seen in the control was observed.

All sputum specimens were examined for acid-fast bacilli and the concentrates were cultured on three media (Löwenstein, modified Dubos, and Tarshis blood agar). Susceptibility tests for cycloserine were made on cultures obtained prior to the initiation of therapy and on all subsequent isolations. These susceptibility tests were made according to the technique outlined in the 1955 revision of *Tuberculosis Laboratory Methods*[5] except that Herrold medium, as modified by Robinson and associates,[6] was used. The results are summarized in table 4.

A total daily dosage of 1.0 gm. of cycloserine, given orally in four divided doses of 250 mg. each, was given to all patients included in this series. At this level the average total daily dose of cycloserine was 16.2 mg. per kg. of body weight and, depending upon individual difference in size, the range in dosage was from 10 to 20 mg. per kg. In an effort to keep psychological bias to the minimum, the drug was administered to the patients as a routine therapeutic measure without undue preliminary discussion.

* * *

Cycloserine therapy resulted in marked symptomatic improvement in all of the 10 patients so treated and the response was most noticeable in the sickest patients. All of the 10 patients noted increased appetite and well-being; 8 of them showed weight gains ranging from 2 to 26 pounds. Weakness and fatigue decreased in most instances within two to four weeks after cycloserine therapy was begun. Among the 10 patients in the series, cough and expectoration were sharply decreased in 6, unchanged in 2, and there were 2 patients in the series who had never had these complaints. These results are summarized in table 1. All of the patients became more alert and cooperative, and this effect again was most noticeable among those who were most toxic at the onset of cycloserine therapy.

Serial chest roentgenograms obtained before and throughout the course of cycloserine therapy were carefully evaluated, and the results are summarized in table 5. On the basis

[5] Tr. Fourteenth Conference on Chemotherapy, Veterans Administration, Army, and Navy, 1955, p. 509.

[6] ROBINSON, J. H., *et al.*: Am. Rev. Tubere., 1950, *62*, 484.

TABLE 5

CHANGES IN CHEST ROENTGENOGRAMS OBSERVED AFTER SIX MONTHS OF CYCLOSERINE THERAPY

	Case Number*	Total
No change	6	1
Mild	2, 5, 8, 9, 10	5
Moderate	1, 4	2
Marked	3, 7	2

* This refers to the numerical order of the cases shown in table 1.

of the usual roentgenographic criteria, 2 patients showed marked improvement, 2 moderate improvement, 5 mild improvement, and in one case no detectable change was apparent. None of the patients showed roentgenographic evidence of progression of their pulmonary tuberculosis while on cycloserine therapy. It should be pointed out that 9 of the 10 patients in the series had extensive chronic pulmonary tuberculosis of such degree that they were not expected to show dramatic roentgenographic improvement.

Bacteriologic studies of the sputum obtained before and throughout the course of cycloserine therapy are summarized in table 3. It may be seen that the sputum of only one patient became negative on microscopy and culture during cycloserine therapy and, in this instance, moderately advanced pulmonary disease of such degree was present that this result might have occurred on bed rest alone. In the remaining 9 patients, tubercle bacilli disappeared from the sputum of 3, as determined by microscopy, but could be detected by culture; and in 6 the bacilli could be detected by both methods. The number of acid-fast bacilli in the sputum decreased in several patients, and this may have been related to the reduction in volume of sputum which was observed. Thus, it was clearly shown that, although the individual patients gained weight and felt much better while on cycloserine therapy, the bacteriologic response lagged behind the clinical improvement.

Cultures of tubercle bacilli obtained before and during the course of therapy were routinely tested for their susceptibility to cycloserine. In the 9 patients who remained infectious throughout the course of the study, there was a definite and gradual rise in the resistance of the bacilli to cycloserine when tested by *in vitro* methods. Two of the original pretreatment cultures were resistant to 5 γ of cycloserine per ml. and the rest were susceptible to this or lower concentrations. After more than 120 days of cycloserine therapy, sputum cultures from all of the patients treated had increased significantly in resistance to the drug. At this time, 2 patient-strains were resistant to 20 γ of cycloserine per ml.; 4, to 10 γ per ml.; and 3, to 5 γ per ml. This acquisition of resistance was slow and steady, beginning within one week after therapy with cycloserine was begun and gradually rising therafter. These observations are summarized in table 4.

Toxicity: Repeated complete blood counts failed to reveal any evidence of bone marrow toxicity which could be attributed to cycloserine. However, 4 patients manifested slightly elevated leukocyte counts ranging from 10,000 to 14,000 per cu. mm., associated with normal differential percentages, and in all instances the leukocytosis disappeared with continued drug therapy. Serial reticulocyte counts were, in all instances, within normal limits.

One patient developed typical grand mal seizures during the course of cycloserine therapy at a time when he was in major respiratory distress, coincidental with pulmonary edema, and required constant oxygen therapy. After effective treatment of the pulmo-

nary emergency, cycloserine therapy was reinstituted with no further difficulty. All of the other serial laboratory tests were normal throughout the course of treatment and there were no depressions of hepatic or renal function in any of the patients.

* * *

The combination of chronic remitting and exacerbating infection with marked variations in host response and adaptation is an essential feature of human pulmonary tuberculosis. This has made it difficult to judge the specific short-term prognosis of any individual on any form of treatment when first seen. These basic characteristics of tuberculosis demand that clinical studies dealing with the efficacy of specific drug therapy must be carefully designed to provide valid support for any conclusions which may result.

It is true that, if progression of the disease uniformly occurs when a specific drug is given while the patients are receiving the best possible supportive care, it is quite easy to be sure that the drug used has no therapeutic efficacy. However, if the improvement is to be credited to the specific therapy, it must be of such degree that it can be clearly attributed to the beneficial effects of the drug employed and not be explained upon the basis of the general influence of bed rest and improved nutrition. This is a difficult judgment to make unless the therapeutic response is most clearly defined and, in a sense, of the "all or none" character. Therefore, any new drug developed for this purpose must, of necessity, be tested: first, for its specific effect against tuberculosis; second, in comparison with standard regimens already available which utilize the above mentioned agents; and, finally, in various combinations with streptomycin, para-aminosalicylic acid (PAS), or isoniazid to determine optimal effect.

The present study was designed in an attempt to determine whether cycloserine had a demonstrable and specific antituberculous effect when used as the sole form of therapy. Limitations of supply prevented this study from being of sufficient size to give a definite answer, but it is believed that, when the results of the study are pooled with those currently being developed by other agencies, it should be possible to determine whether or not cycloserine therapy offers sufficient promise to warrant further investigation.

The results of the present study were, in one sense, encouraging in that all of the patients who received cycloserine improved clinically and the previous progression of their disease was inhibited. However, careful bacteriologic study gave less encouraging results and in only one instance did the sputum cultures become negative for tubercle bacilli. This reversal of infectiousness occurred in a patient with fresh moderately advanced disease which might well have improved on bed rest alone. This patient (*No. 7*) had a segmental resection of a solid residual lesion after 180 days of cycloserine therapy, and the surgical specimen was found to be positive for acid-fast bacilli on microscopy. Cultures of the resected specimen, however, failed to reveal any growth of tubercle bacilli after three months' incubation. The remaining 9 patients continued to have sputum positive for tubercle bacilli on culture and only 3 of them had sputum negative on microscopy.

It is noteworthy that resistance to cycloserine developed quickly and gradually in the strains from all of the patients who remained infectious during the course of study. In 2 of the patients, the drug resistance of the tubercle bacilli probably had reached a limiting level and in 4 others it was significantly high. This finding suggests that resistance would become a limiting factor in many cases if cycloserine were to be used alone. The gradual acquisition of resistance, however, suggests that combination with other drugs might well retard its appearance.

The changes that were noted on serial chest roentgenograms also were in the general direction of improvement, but in no instance were they as striking as the gain in weight

and well-being that these patients showed. It should be admitted, however, that 9 of the patients in this series had far advanced chronic pulmonary tuberculosis of a marked degree. Therefore, in these cases, any demonstrable improvement in the chest roentgenogram must assume some significance.

The present writers believe that cycloserine offers some promise in the therapy of pulmonary tuberculosis. However, its specific antituberculous effect seems to be much less than that of either streptomycin or isoniazid and more comparable with that of PAS. For this reason, as well as the observation that resistance to cycloserine emerges soon after therapy with the drug is started, it is believed that it should not be used routinely as the sole agent in the treatment of tuberculosis. Subsequent studies may well demonstrate more clearly the precise role of cycloserine in combined therapy with streptomycin or isoniazid. It is possible that cycloserine may have particular value in the treatment of toxic and acutely ill patients by some type of action not clear at present.

In summary: A series of 10 cases of pulmonary tuberculosis treated with 1.0 gm. of cycloserine per day is reported. Clinical improvement was marked in all cases. Chest roentgenograms demonstrated improvement in 9 of the 10 patients, being marked in 2, moderate in 2, and mild in 5. No instances of progression of disease were noted during the course of therapy. Bacteriologic studies revealed less satisfactory results as the sputum cultures became negative for tubercle bacilli in only one patient; in only 3 patients did the sputum become negative on microscopy. Drug-susceptibility studies showed that cycloserine resistance was gradually acquired, being demonstrable within thirty days after the initiation of therapy. In 2 patients, the resistance of the strains rose to probable limiting levels and in 4 patients it became significantly high. Epileptic seizures occurred in one patient while he was in acute respiratory failure; no other significant instances of toxicity were observed. It is concluded that cycloserine has a specific antituberculous effect, but that it should not be used as the sole agent in the treatment of tuberculosis. Further study is needed to evaluate the role of cycloserine in combined therapy with streptomycin and isoniazid.

Suburban Cook County Tuberculosis
Sanitarium District
Hospital-Sanitarium
Hinsdale, Illinois

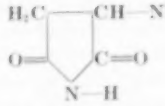
William Lester, Jr.[1]
Alexander Salomon
Arthur F. Reimann
Eli Shulruff
George S. Berg

January 3, 1956

[1] The writers wish to thank Miss Rosemary Colton for her excellent technical assistance in the bacteriologic studies cited in this report.

CLINICAL, BACTERIOLOGIC, AND PHARMACOLOGIC OBSERVATIONS UPON CYCLOSERINE[1]

Less than a year ago a new antituberculous agent was introduced for clinical use. At least three pharmaceutical companies have described the agent almost simultaneously as D-4-amino-3-isoxolidone (cycloserine[2]), although different proprietary names have been assigned to it, such as Oxamycin® and Seromycin®. In the past nine months independent investigators have isolated, crystallized, and characterized the material.[3-6] Its chemical structure is:



The present report summarizes certain *in vitro* studies and observations made on 8 adults with tuberculosis who received the drug continuously for six to eight months. In brief, cycloserine shows definite action in interrupting the growth of tubercle bacilli *in vitro* and *in vivo*. It has been shown to have some action in altering favorably the course of human tuberculosis with a minimal, although definite, degree of toxicity. It is not as powerful an agent in these respects as either streptomycin or isoniazid alone.

In vitro observations: Two strains of human tubercle bacilli (H37Rv) and two nonpathogenic acid-fast bacilli (*M. ranae* and H37Ra) were grown in three standard media and their growth as altered by varying concentrations of cycloserine was observed. The human strains of tubercle bacilli were streptomycin-PAS-isoniazid resistant and streptomycin resistant-isoniazid susceptible. Liquid Proskauer and Beck medium, liquid Tween®-albumin medium, and American Trudeau Society potato-egg solid medium were used. The organisms had been maintained alternately in Proskauer and Beck and American Trudeau Society media prior to the present studies, and one of the virulent strains had been isolated initially from a patient with pulmonary tuberculosis.

In Proskauer and Beck medium, using about 100,000 organisms as the inoculum, 3 to 6 γ of cycloserine per ml. inhibited completely the growth of four strains tested; 10 to 20 γ in liquid Dubos or American Trudeau Society media were required for the same degree of inhibition. The results of the drug-susceptibility tests, however, were directly proportional to the inoculum size of the organisms. The larger the inoculum, the greater the concentration of drug which was required for inhibition. By increasing the number of viable organisms from approximately 100,000 to more than 1,000,000, the end point of susceptibility increased from three to five times. For example, in liquid Tween-albumin medium, the lesser number of H37Rv organisms gave an end point of 2 to 5 γ per ml.; the greater number, 10 to 20 γ. This degree of variation was observed in all media and with the four strains measured, although it was least apparent with Proskauer and Beck medium.

[1] The laboratory work in this study was supported by grants from Charles Pfizer & Co., Inc., Brooklyn, New York; and the Onondaga Health Association, Syracuse, New York.

[2] Cycloserine was generously supplied by Dr. R. M. Rice, Medical Director, Eli Lilly and Company, Indianapolis, Indiana.

[3] Conzelman, G. M.: Antibiot. & Chemother., 1955, *5*, 444.

[4] Harris, D. A., *et al.*: Antibiot. & Chemother., 1955, *5*, 183.

[5] Harned, R. L., *et al.*: Antibiot. & Chemother., 1955, *5*, 204.

[6] Welch, H., *et al.*: Antibiot. Med., 1955, *1*, 72.

TABLE 1

DETERIORATION OF CYCLOSERINE AT INCUBATOR TEMPERATURES

Initial Concentration	Egg and Potato Medium (ATS)	Concentration After One Week Incubation γ *per ml.*	
		Liquid Tween-Albumin Medium	Proskauer-Beck Medium
γ *per ml.*			
10	0.4	0	0
20	12.5	0.4	0.4
40 to 50	46.4	36.0	30.4
100	89.6	66.4	66.4

In the final interpretations of drug susceptibility, the timing of the end point determination offered another variable. There was a significant change in the results observed at four and at six weeks. This probably was dependent upon deterioration of the drug at incubator temperatures of 34° to 37°C. It might also have reflected the increasing number of organisms in the medium as the culture became older.

Cycloserine is not a tuberculocidal drug, for sterilization in any medium was not achieved with the organisms studied, dosage of drug used, or extent of exposure of drug and organism. Cycloserine is a reasonably stable drug under most laboratory conditions[4, 5] but does deteriorate appreciably at incubator temperatures. A loss of potency, from five- to tenfold in the lower concentrations, was noted after one week in both Proskauer and Beck and Tween-albumin media (table 1). This defect interferes with interpretation of the drug-susceptibility tests.

Observations on the emergence of strains of tubercle bacilli resistant to cycloserine upon prolonged exposure *in vitro* have not been completed. It would appear that such a phenomenon does occur.

Observations in animals: The distribution of cycloserine in blood, tissue, and urine has been measured in 4 rabbits. In general, following either oral or parenteral administration, the absorption, distribution, and excretion of cycloserine are essentially the same, with the parenteral route resulting in slightly higher serum concentrations. All of these determinations were made by a modification of the colorimetric method of Jones, using a Beckman spectrophotometer to record the final color density.[7]

Peak blood concentrations appeared promptly after administration, and effective concentrations extended for eight hours (figure 1). Distribution of the drug to liver, lung, and kidney was measured. It appeared promptly and after the second hour there was no unusual concentration in any tissue studied (figure 1). The urinary excretion was rapid, and after a single dose approximately 75 per cent was recovered in the urine within twenty-four hours. Toxicity to the drug was not encountered in any animal which received a dose of 75 mg. per kg. of body weight—an amount five times greater than is generally used in humans. The effects of cycloserine on experimental tuberculosis in animals were not studied.

Pharmacologic observations in the human: The cycloserine concentrations in the blood and cerebrospinal fluid of adult subjects were determined following single or multiple oral doses. The standard single dose administered was 0.5 gm. and, in those who received the drug continuously, 0.5 gm. twice daily (approximately 12 to 15 mg. per kg.) was prescribed.

[7] GRIFFITH, R. S.: Personal communication.

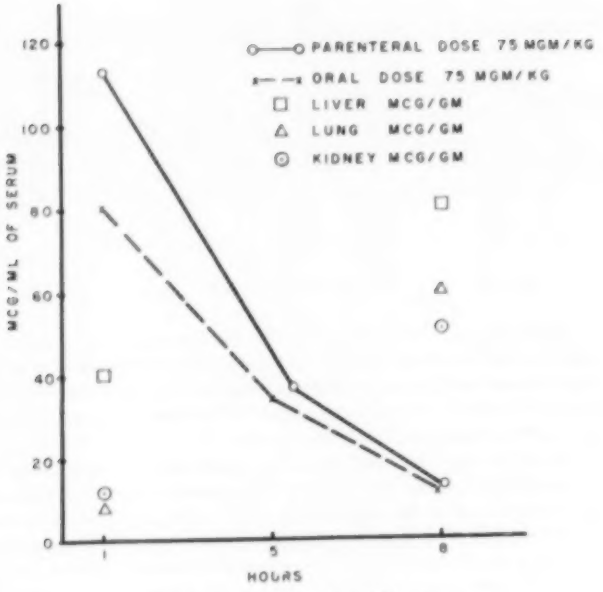


FIG. 1. Cycloserine in rabbit tissue

Cycloserine appeared promptly in the cerebrospinal fluid. Lumbar taps were performed upon 3 subjects: 2 had no meningeal disease; the other was recovering from tuberculous meningitis. One and six hours after a 0.5 gm. dose, cycloserine concentrations of 40 and 8 γ per ml. of cerebrospinal fluid were found. The concentrations in the cerebrospinal fluid thus are in the therapeutic range. They appeared promptly after a single dose, and there was not an appreciable blood-brain barrier.

Blood concentrations of cycloserine were determined both after a single dose and following continuous daily doses. Figure 2 is a composite average curve of serum concentrations following a 0.5 gm. dose in 8 patients. Single doses of the drug were given at 8:00 a.m. and 6:00 p.m. The thin vertical lines at each hour indicate the "spread" among the group, there being from four to ten separate determinations at each hour during the four months in which the drug was being administered. The peak concentration was attained within three to four hours, and therapeutic concentrations of more than 10 γ were maintained constantly for at least sixteen hours daily. The drug concentrations between the sixteenth and twenty-fourth hours each day were determined too infrequently to be included in the figure. The few which were measured indicated that concentrations of more than 10 γ persisted twenty-four hours every day. There was no cumulative effect.

Urine excretion was rapid and fairly complete; from 50 to 80 per cent of the daily dose of cycloserine was excreted within twenty-four hours.

Concentrations of drug within diseased tissue were not measured.

Clinical observations in the treatment of chronic pulmonary tuberculosis: Eight patients with bacteriologically confirmed pulmonary tuberculosis of varied extent were treated continuously with cycloserine, 0.5 gm. twice daily orally for six to eight months. All had

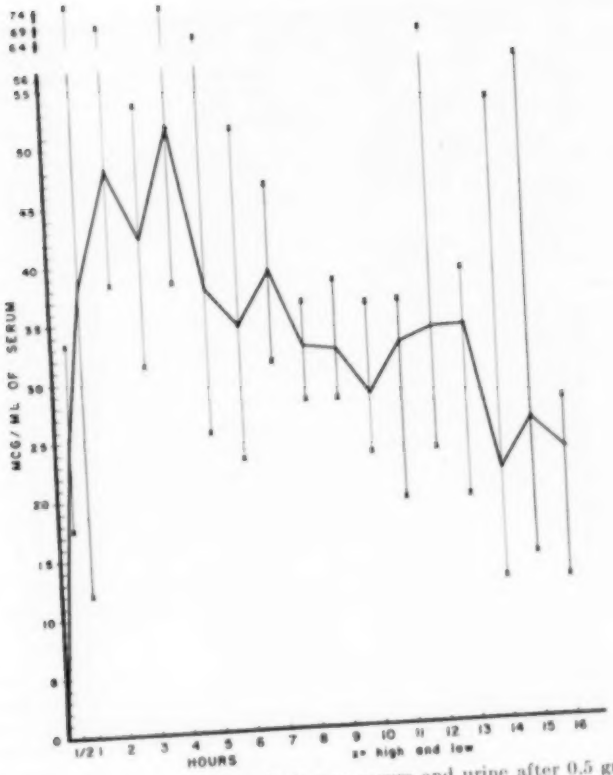


FIG. 2. Cycloserine concentrations in human serum and urine after 0.5 gm. oral dose twice daily. Average daily urinary excretion: 0.3 to 0.68 gm.

far advanced pulmonary disease with cavitation and, in addition, one of them had chronic renal tuberculosis. Five patients had received streptomycin, isoniazid, and PAS, either alone or in various combinations for periods of one to thirty months, and their tubercle bacilli were resistant to all three agents. Of these 5 patients, cycloserine was given to one in addition to isoniazid; to 2 patients, in addition to isoniazid-PAS; and to 2 patients, in addition to isoniazid-streptomycin. The remaining 3 patients had never received antituberculous drugs and cycloserine was administered as single-drug therapy.

Observations made over a six- to eight-month period have shown that a reduction has occurred in both the volume and bacillary content of the sputum in all patients. Nevertheless, there has been no reversal of infectiousness. Four of the 5 patients on previous long-term drug therapy have shown no significant roentgenographic change during the period of therapy with cycloserine. The other showed a reduction of 1 cm. in the diameter of a 4 cm. thin-walled cavity. The 3 patients who received only cycloserine have all shown some roentgenographic clearing, as well as complete loss of fever and some gain in weight. The patient with renal tuberculosis failed to demonstrate objective evidence of improvement in either his pulmonary or renal disease.

All patients had monthly determinations of erythrocyte count or hematocrit, leukocyte count with differential, complete urinalysis, blood nonprotein nitrogen, phenolsulfonphthalein excretion, bromsulfphthalein excretion, and cephalin flocculation, and/or thymold turbidity tests upon serum. No abnormalities were shown by any one of these measurements. One patient had an epileptiform seizure on the eleventh day of therapy. The cycloserine concentrations in the blood were more than 40 γ per ml. at the time. The drug was stopped for four days, was then reinstituted in gradually increasing dosage (until 1 gm. per day was used), and no further convulsions occurred. The patient's encephalogram was described as a bilateral cerebral dysrhythmia consistent with, but not diagnostic of, epilepsy. In one of the 3 other patients who had electro-encephalograms taken prior to therapy and at the four-month observation point, there was also a change to an abnormal pattern in the electro-encephalogram.

Shortly after commencing therapy, i.e., within twenty-four to forty-eight hours, 5 of the 8 patients complained of a variety of central nervous system symptoms, ranging from depression to marked elation. In one, the sensation was described as similar to her often-experienced feelings stimulated by excessive amounts of alcohol. Within a week or so these complaints subsided and therapy was not discontinued. One patient, not included among the 8, refused the drug after the fourth day of his therapy; it had produced a significant degree of excitement, and he feared for his health because of it. As he was known to be unstable psychically, continuation of therapy was not encouraged. Five patients also experienced a slight elevation of temperature during the initial few days of therapy. Elevations ranged from 1.5° to 3°F., but the fever fell spontaneously in all.

Because the clinical, roentgenographic, and bacteriologic responses were definite, although not impressive in any instance, only a single case report is described. The response of this patient was the best observed.

J. G., a 31-year-old white, alcoholic male, was admitted to the Syracuse Veterans Administration Hospital on April 14, 1955, with a chronic cough which had been present for at least five years. During all of this period, his dietary habits had been poor. In the several months before admission he had had night sweats and an increase in the nonproductive cough. For at least three weeks prior to hospitalization he had noted marked dyspnea on exertion and dependent edema.

Examination revealed a chronically ill, dyspneic man with a temperature of 99.2°F.; pulse, 84 per minute; respiration of 26 per minute; and blood pressure of 160 systolic, 80 diastolic in mm. of mercury. Bilaterally dullness and numerous rales from the apex to the fifth dorsal vertebra posteriorly were observed. The heart was enlarged to the left. The pulmonic second sound was greater than the aortic second sound. The liver was palpable 3 cm. below the costal margin and was tender. The left epididymis was greatly enlarged, nodular, and tender, and the prostate was slightly enlarged. The patient's legs were markedly edematous. His sputum was repeatedly positive for tubercle bacilli on microscopy and culture. Hemogram, urinalysis, phenolsulfonphthalein, and liver function studies were normal. An electrocardiogram showed inverted T waves on leads V2, 3, and 4, but was otherwise unremarkable. An initial roentgenogram of the chest showed cardiac enlargement and patchy irregular areas of infiltration involving the upper one half of both lung fields. Laminagrams did not demonstrate definite evidence of cavitation. Diagnoses of advanced active tuberculosis and beriberi heart disease with congestive failure were made.

Initial treatment consisted of an 800 mg. sodium diet, bed rest, and daily parenteral administration of 100 mg. of thiamine chloride. In the first ten days of hospitalization, the patient lost 25 pounds, all of edema fluid. A chest roentgenogram which was taken five days after the initial one (figure 3) showed a return of cardiac size to normal but no change in the parenchymal infiltration. One day later (April 21) administration of cycloserine was

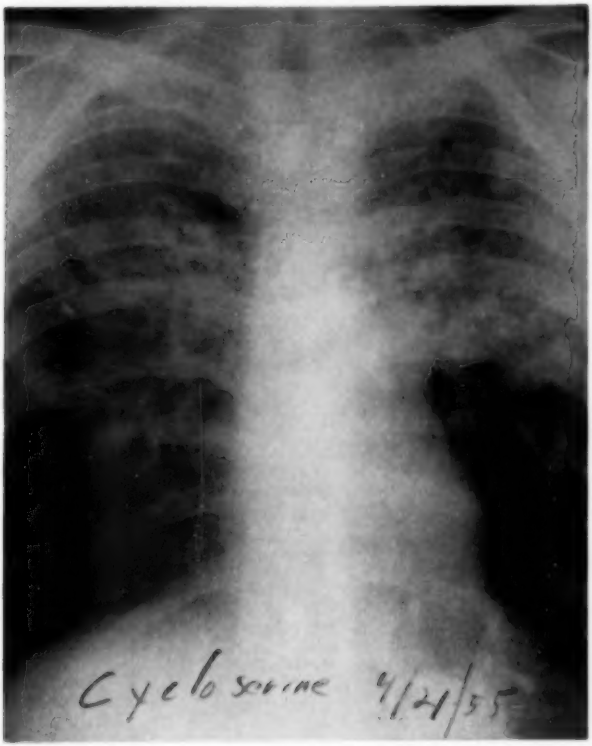


FIG. 3. Roentgenogram of J. G., 31-year-old white male, taken five days after hospital admission. Evidences of the initial extensive heart failure have subsided and the heart size has diminished markedly in the five days. Heart failure was relieved with thiamin but not digitalis. Film reveals the advanced bilateral tuberculosis.

started, 500 mg. twice a day, orally. Eleven days later (May 2) he had a generalized convulsion, with biting of the tongue. Cycloserine was stopped for four days. It was reinstituted in a dosage of 250 mg. twice daily for ten days; thereafter 250 mg. daily, three times daily, for twenty days; and finally 500 mg. twice daily, for the remainder of the four-month hospital stay.

The patient remained afebrile and, except for some improvement in appetite and a 6 pound gain of real weight, showed no significant change in symptoms during hospitalization. Another chest roentgenogram obtained four months later (figure 4) showed moderate clearing of the pulmonary infiltration bilaterally. An electro-encephalogram at this time showed two short episodes of "spiking" at a 5-per-second rate, but was otherwise normal. Repeated laboratory examinations showed no evidence of abnormality in hematopoietic, renal, or hepatic functions.

In summary: Cycloserine has definite *in vitro* activity against tubercle bacilli in a range of concentrations low enough to be attained in human blood. It has tuberculostatic rather than tuberculocidal activity. Observations *in vitro* indicate that it is a less active drug

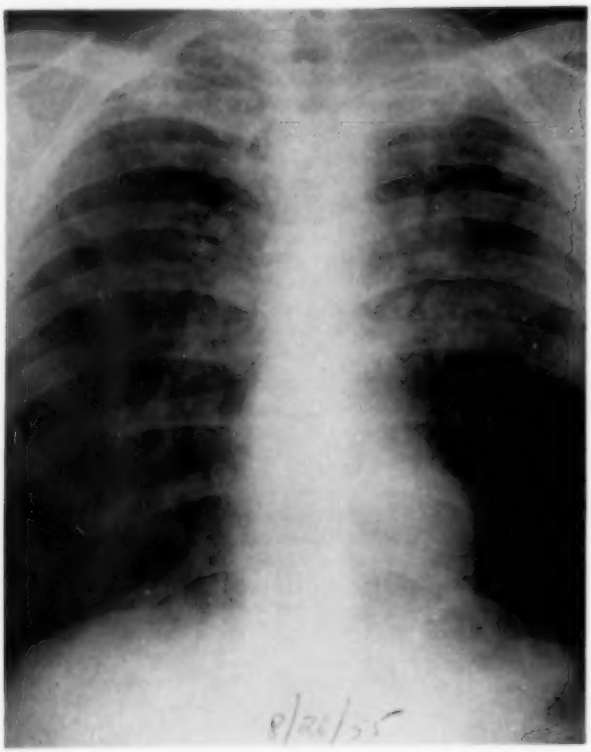


FIG. 4. Roentgenogram obtained four months after figure 3, showing improvement. The patient's sputum is still positive for tubercle bacilli and his symptoms have not changed appreciably.

against tubercle bacilli than either isoniazid or streptomycin.[8, 9] Tubercle bacilli have the potential of emerging resistant to the drug after prolonged exposure.

Pharmacologically, cycloserine is absorbed easily and rapidly from the gastrointestinal tract, is distributed to lung, kidney, and brain in adequate concentrations (at least in the rabbit), and is excreted promptly in urine.

It has not been demonstrated that cycloserine is as effective as either isoniazid or streptomycin in tuberculous adults with far advanced pulmonary disease. It is active against streptomycin- and isoniazid-resistant tubercle bacilli and, therefore, may have limited application in patients with tuberculosis produced by such organisms.

On occasion cycloserine has a clear, unexplained central nervous system toxicity leading to the production of convulsions, which makes the drug hazardous. The toxicity is not serious enough, however, to preclude its prescription with the proper indication.

[8] CUMMINGS, M. M., *et al.*: Antibiot. & Chemother., 1955, *5*, 198.

[9] EPSTEIN, I. G., *et al.*: Tr. Fourteenth Conference on Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1955, p. 325.

Other toxicity studies in man have demonstrated that, with 1 gm. daily doses given for as long as eight months, no adverse effects upon kidney, liver, or bone marrow develop.

Cycloserine is another antituberculous drug which may become a valuable tool in the therapy of the human disease. Virtually all evidence points to the conclusion that its activity is less than that of either streptomycin or isoniazid. More studies are warranted, before final definition of its usefulness can be estimated (including its use with either streptomycin or isoniazid), to determine its effect upon the emergence of drug-resistant variants.

Department of Medicine
State University of New York
Upstate Medical School
Syracuse, New York

The Onondaga Sanatorium
Syracuse, New York

Veterans Administration Hospital
Syracuse, New York

A. D. Renzetti[10]
K. W. Wright
J. H. Edling
Paul Bunn

[10] With the technical assistance of Frances Robinson and Donald Johnson.

TUBERCULOUS ADENITIS

Twenty-Three Cases Treated with Isoniazid Alone

The Pulmonary Clinic of the Out-Patient Department of The New York Hospital has been following patients with tuberculous adenitis who have been treated with isoniazid alone. The patients have been treated in the Out-Patient Clinic from the beginning of their therapy following hospital discharge. In a few instances the patients have had previous sanatorium care.

Drug therapy has been uniformly long-term in character. The amount of complete rest has been less uniform due to the extent of disease, age difference, and social and economic circumstances. With one exception, the patients were not allowed just to "take it easy" or stay at work. Every effort has been made to ensure a maximal amount of rest in the belief that tuberculous adenitis is a systemic disease and exists as a subacute or chronic form of lymphohematogenous tuberculous infection. Instances of a localized form of tuberculous adenitis in the cervical region, when the port of entry has been the oropharynx, presumably occur. Nevertheless, many case histories bear out the fact that even cervical adenitis may follow the involvement of mediastinal and tracheobronchial nodes following a primary pulmonary complex. Thus it is that often the clinician is dealing at the same time with: an unhealed primary focus, hidden foci of which the involved lymph node is but one manifestation, and probable distant foci unknown and unheralded.

From 1949 to 1953, 11 patients with cervical adenitis were treated with a short-term course of streptomycin or dihydrostreptomycin. Two were well after three years, 2 were well after two years. There were no follow-up observations on 2 patients, but in 5 the disease relapsed with the appearance of pulmonary lesions. One of these patients whose infection relapsed after short-term chemotherapy with streptomycin is included in the present series. The disease has not relapsed in a two-year follow-up period after conclusion of an eighteen-month course of isoniazid. At about the time it became apparent that short-term streptomycin therapy was not adequate in the treatment of adenitis, isoniazid became available. This was selected for long-term therapy instead of streptomycin–PAS because of its simplicity of administration and because of the fact, previously demonstrated in this Clinic,[1] that even in generalized miliary tuberculosis, long-term treatment with isoniazid alone was remarkably effective. In addition, as it was not known to what extent surgery might be necessary in conjunction with long-term chemotherapy, by the plan of single-drug therapy, streptomycin–PAS could be reserved to cover any subsequent surgical need. Isoniazid therapy was combined with the best supportive treatment practicable in each individual circumstance.

Twenty-three patients have thus been treated. Five were male, 18 were female. Nine were white and 14 were non-white. The age distribution was wide with the highest incidence in the twenty- to forty-year age group as follows:

Less than 20 years	4 patients
20 to 29 years	6 patients
30 to 39 years	9 patients
40 to 49 years	2 patients
50 to 59 years	2 patients

[1] Clark, C., *et al.*: Am. Rev. Tubere., 1952, *66*, 391.

TABLE 1

LOCATION OF LESIONS IN TWENTY-THREE PATIENTS

Location of Lesion	Number of Patients	Location of Lesion	Number of Patients
Unilateral cervical	6	Suprasternal, submandibular cervical	1
Unilateral axillary	1	Supraclavicular, cervical, and skin	1
Unilateral cervical and axillary	1	Cervical and subauricular	1
Unilateral perihilar	2	Supraclavicular and subauricular	1
Unilateral cervical and hilar	1	Submandibular and sinus tract	1
Supraclavicular	3	Mesenteric	1
Supraclavicular and unilateral mediastinal	1	Inguinal with sinus tract	1
Bilateral mediastinal	1		

The location of lesions also showed wide distribution and variableness, as may be seen in table 1.

Previous tuberculous disease: In 15 patients, no previous tuberculous disease had been recognized. Of the remaining 8, 7 had had earlier episodes of adenitis. In one patient this had occurred thirty-four and twenty-eight years before; in another ten and one year previously. In others the interval was from one to five years.

There were only 2 patients who had had active pulmonary disease. One patient had been ill twenty-nine years ago and had been well in the interval. The second patient had had pleurisy with small effusion one year before simultaneously with the appearance of the inguinal node which subsequently ruptured and which brought her to the Clinic.

Concurrent active tuberculosis: There was no active pulmonary disease demonstrable by roentgenography in any of the 23 patients. Two had bone erosion in the cervical spine and one had a questionable kidney lesion, as was demonstrated by an intravenous pyelogram. There was one patient with a gastric culture positive for tubercle bacilli without roentgenographic evidence of pulmonary disease. The same patient had a positive urine culture for tubercle bacilli, and an intravenous pyelogram was suggestive of involvement of one kidney.

Diagnostic criteria: A lymph node was biopsied in each patient and, when possible, the excised material was cultured. Gastric washings and urine were likewise cultured for tubercle bacilli. The tuberculin reactions and erythrocyte sedimentation rates were obtained in all cases.

Four factors were taken into consideration in establishing diagnosis: (*1*) the clinical picture; (*2*) morphology of nodes showing caseation, epithelioid cells, Langhans' giant cells, and tubercle formation; (*3*) positive tuberculin reactions; (*4*) culture of nodes and aspirations positive for tubercle bacilli. Not all patients met all criteria. Twenty patients met the first three criteria and 5 of these patients also had cultures positive for tubercle bacilli on aspiration or biopsy of the lymph nodes. The remaining 3 patients had large inaccessible nodes, and the removal of scalene and cervical nodes was not helpful. It was believed that the clinical findings, together with the positive tuberculin tests warranted the start of therapy. Prompt regression of the nodes after starting isoniazid was regarded as sufficiently confirmatory to warrant the inclusion of these cases in the series.

Hospital and sanatorium care: There was no fixed policy with respect to hospitalization or to the duration of hospital or sanatorium care when the latter was effected. In all cases

rest therapy was prescribed but, because of various social and personal factors, it was usually not possible for this to be carried out in the form of prolonged hospital or sanatorium residence. The duration of hospitalization is shown in table 2.

Patients not hospitalized had diagnostic procedures carried out on an ambulatory basis in the Clinic. When the diagnosis was established a rest regimen was instituted with but one exception. Six of the 23 patients were placed in sanatoriums.

Drug therapy: As has been stated, therapy was limited to isoniazid. The dosage used was 5 mg. per kg., which means that the usual dosage was 200 to 300 mg. a day in three divided doses. The length of time during which isoniazid was given is shown in table 3.

Response to therapy: One patient has been lost to follow-up observations. With two exceptions, the response to chemotherapy was good. The effectiveness of the isoniazid therapy was measured by healing of sinus tracts, loss of induration, and regression of the nodes in size and tenderness. In general, marked improvement was noted during the first three months of therapy. Within six months, the lesions were apparently arrested in all but one case. This patient had extensive involvement together with concomitant disease of a different nature and it was nine months before the sinus tract closed. All patients had a markedly improved sense of general well-being.

There were 2 patients in whom the adenitis relapsed. In a young Negro woman with extensive mediastinal involvement and a lesion of a cervical vertebra, there was prompt response to the drug, as evidenced by regression of the nodes on roentgenographic examination. After fifteen months of therapy, the mediastinal nodes increased to the original size. Streptomycin was added to the regimen, with good response.

The second patient was a 23-year-old Negro children's nurse admitted to the surgical service of The New York Hospital on January 25, 1953, because of a lump in the neck, pres-

TABLE 2

DURATION OF HOSPITALIZATION OF TWENTY-THREE PATIENTS

Duration of Hospitalization	Number of Patients
None	8
Less than one month	5
4 weeks	3
6 to 8 weeks	4
12 weeks	1
6 months	1
9 months	1

TABLE 3

DURATION OF ISONIAZID THERAPY FOR TWENTY-THREE PATIENTS

Duration of Therapy	Number of Patients
months	
6 (patient left without permission)	1
7 (relapsed)	1
12	4
15 (relapsed)	1
18	12
24	4

ent for six months. This had been asymptomatic until two months before admission when the patient had had choking sensations, chills, and fever to 101° F. for several days. Symptoms subsided and the patient had slight difficulty in swallowing thereafter. On admission she did not appear acutely or chronically ill. Her temperature was 100.4°F. On physical examination there was a mass 2 by 2 cm. to the left of the trachea, above the clavicle which was firmly fixed and nontender. A roentgenogram of the chest showed a widening of the mediastinum on the right (figure 1). There was a leukocytosis of 16,000.

A biopsy showed the mass to be a supraclavicular lymph node. On section this was typical of tuberculosis with caseous necrosis. An aspiration biopsy culture was reported positive for tubercle bacilli thirty-nine days later. The patient was transferred to the pulmonary service where isoniazid was started on February 9. Within two weeks the temperature dropped to a normal range as did the leukocytosis. Within one month there was a marked reduction in the size of the mediastinal mass. Improvement continued during the four months of hospital stay. The patient was allowed home to await sanatorium care, which she later refused. She was followed in the Clinic and continued on the drug without much control of her care. In September, seven months after the start of therapy a pulmonary lesion was observed (figure 2). Hospitalization was resumed and streptomycin-PAS were given, with benefit.

Patients were allowed to resume activity gradually after nine to twelve months and full-time work thereafter. Some continued drug therapy while resuming physical activity. Of the 20 patients followed whose lesions did not relapse, the majority are receiving no chemotherapy and are at school, working at home, or at their usual occupations (table 4).

* * *

Twenty-three patients with tuberculosis of the lymph nodes have been treated with isoniazid as the sole therapeutic agent. Treatment was maintained from six to twenty-

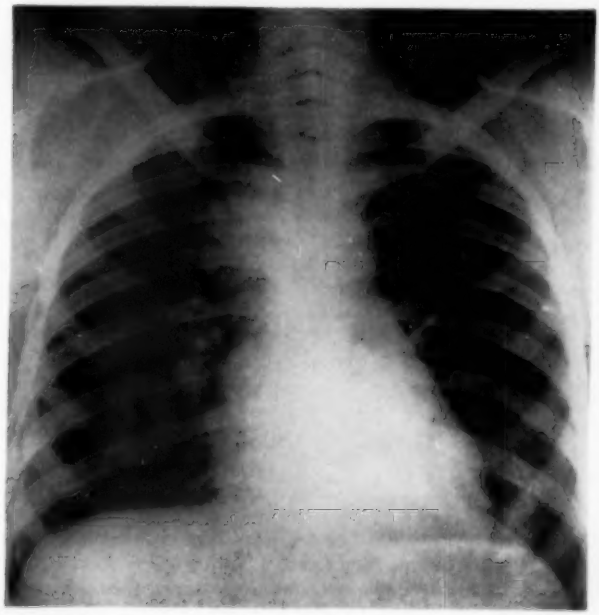


FIG. 1. Roentgenogram obtained on admission to the hospital.

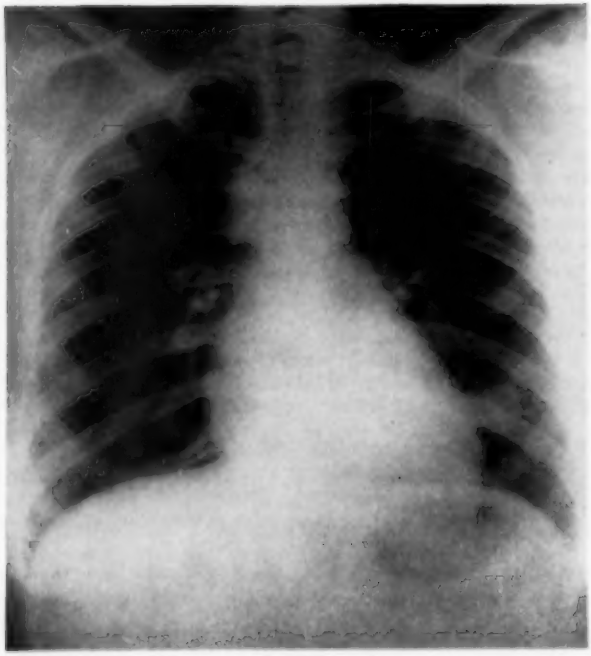


FIG. 2. Roentgenogram obtained after seven months of therapy, showing regression of superior mediastinal enlargement and a well-demarcated density near the cardiac silhouette on the left.

TABLE 4

FOLLOW-UP OBSERVATIONS ON WORK STATUS OF TWENTY PATIENTS

Work Status	Number of Patients
In sanatoriums	3
On chemotherapy and limited activity	1
On chemotherapy and working 6 months	1
Beginning work	1
Working 6 months	4
Working 12 months	3
Working 15 months	1
Working 18 months	4
Working 21 months	1
Working 33 months	1

four months, the majority receiving eighteen months of chemotherapy. Response was prompt in all cases. One patient has been lost to follow-up observation. In only 2 patients did the lesion relapse or new foci appear before the intended treatment was completed. None of the 15 patients who received twelve to twenty-four months of treatment have

experienced relapse in six to thirty-three months of follow-up. Five have not completed their course of therapy, but 2 of this number are engaged in normal activity. It is still too early to evaluate the relapse rate, which will determine whether this may be regarded as definitive treatment for most patients with tuberculous lymphadenitis. The early results are encouraging, however, and indicate: that surgical excision of tuberculous lymph nodes is not necessary except in the exceptional instance; and that single-drug therapy with isoniazid is adequate in most instances. Furthermore, the isoniazid is easily administered and conserves other agents in case of exacerbation of the tuberculosis. It is only realistic to recognize that the tubercle bacillus is a persistent and ubiquitous organism in any host, and is not likely to be removed completely by surgery. Hence, when one infection has occurred, an ever present need remains for manifold defenses against it.

Department of Medicine — Katharine Butler
The New York Hospital-Cornell University Medical College
New York 21, New York

December 5, 1955

A CLINICAL TRIAL OF S-ETHYL-L-CYSTEINE IN PULMONARY TUBERCULOSIS[1]

Beginning in January, 1954, S-ethyl-L-cysteine was made available to the writers for clinical trial in pulmonary tuberculosis[2] because of its promising therapeutic effects in experimental tuberculosis of mice.[3] Through 1954 and 1955, 23 patients have received significant amounts of this substance and, although therapeutic results were not spectacular, it seems advisable to report these studies briefly for possible guidance of future investigators with similar preparations. Clinical interest in this compound arises from the fact that it represents an entirely new group of antituberculous substances, unrelated to any previously reported material used in clinical medicine.

At the beginning of the study it was necessary to determine what doses might be tolerated over prolonged periods and to be alert for any toxic manifestations. In addition to clinical observations, numerous laboratory studies were carried out to determine the functional status of such organs as the liver, kidneys, and bone marrow. Complete blood counts, observation of blood cell morphology, reticulocyte counts, urinalyses, blood urea determinations, and estimation of bromsulphthalein dye retention were performed at frequent intervals in all cases while ascending doses of the drug were administered.

In no instance was an abnormality detected which could be attributed to administration of the drug. Finally when a dosage level of 4.0 gm. daily (1.0 gm. four times a day by mouth) was attained no further increases were attempted. Such doses were administered for as long as nine months continuously without producing any manifest injury.

Each patient and his associates complained, often bitterly, about an obnoxious odor to the breath which could be detected at considerable distance. It was a pungent, somewhat sulfurous and garlic-like stench and was rather characteristic of some mercaptans. Because of this source of annoyance patients frequently requested that medication be discontinued. In view of experimental results reported by Solotorovsky and associates, it now appears that this volatile material may be an active therapeutic agent (ethyl mercaptan), possibly the sole active derivative of S-ethyl-L-cysteine, produced by intraintestinal chemical cleavage from bacterial action.[4, 5]

The patients studied have been divided into three groups according to prior treatment, dosage of S-ethyl-L-cysteine, and duration of therapy.

Group I: This category includes 6 patients with far advanced bilateral active pulmonary tuberculosis, all of whom had received prior antimicrobial therapy and in whom there was complete or partial resistance of the tubercle bacilli to streptomycin, isoniazid, and para-aminosalicylic acid. These patients all had sputum positive for acid-fast bacilli on microscopy and they were all treated for four to five months with S-ethyl-L-cysteine. For the first five weeks each patient received 1 gm. daily (250 mg. four times a day), following which they each received 2 gm. daily for the remainder of the course. One patient's sputum became temporarily negative for acid-fast bacilli on microscopy, whereas

[1] This project received financial support through a research grant (E-110) from the National Microbiological Institute of the National Institutes of Health, United States Public Health Service.

[2] S-ethyl-L-cysteine was generously supplied by the Department of Clinical Investigation of Merck and Company, Rahway, New Jersey.

[3] Solotorovsky, M., *et al.*: Am. Rev. Tubere., 1954, *70*, 806.

[4] Solotorovsky, M., *et al.*: Am. Rev. Tubere., 1956, *74*, 72.

[5] Ogiusky, E. L., *et al.*: Am. Rev. Tubere., 1956, *74*, 78.

in 5 other patients the sputum remained positive. The roentgenograms obtained before and after therapy revealed no change in 5 patients and were judged to show progression of disease in one. Erythrocyte, leukocyte, hemoglobin, and reticulocyte estimations remained stable or were slightly improved in all. All patients gained weight. All of these patients ultimately received other therapy following the cessation of S-ethyl-L-cysteine. Of this group 4 are dead, one is terminally ill, and one is alive in the hospital (January, 1956).

Group II: Four patients were treated for seven to nine months with 4 gm. of S-ethyl-L-cysteine daily. Two of these patients had received prior antimicrobial therapy, one of them had a tuberculous adenitis with peritracheal adenopathy. Two of these patients had fresh, bilateral far advanced active pulmonary tuberculosis. One patient had a sputum "conversion," and two sputum "conversion" failures were noted. The roentgenograms showed improvement in 3 cases, including the regression of peritracheal adenopathy, and the roentgenographic findings remained unchanged in one patient. The hemogram remained stable in all. All patients gained weight. No clinical toxic effects were recorded. All patients of this group received therapy of another nature later. Three patients are living and are still hospitalized and the present status of one other is unknown.

Group III: Thirteen patients with newly discovered untreated, far advanced, active pulmonary tuberculosis were treated with 4 gm. of S-ethyl-L-cysteine daily for periods ranging from one and one-half to six months. Eight patients were treated for one and one-half to three months; 5 patients, from four to six months. Eight patients showed some roentgenographic improvement, 4 were unchanged, and in one the disease worsened. The hemogram remained stable or improved in all. All gained weight, and all received other therapy at a later date. Five of these patients are still hospitalized. Of those who received S-ethyl-L-cysteine for one and one-half to three months, the roentgenographic evaluations showed worsening in one, no change in 3, and improvement in 4. Six patients in this therapy range continued to discharge tubercle bacilli and 2 patients attained noninfectious status during the course of therapy. Of those patients who received S-ethyl-L-cysteine, 4 gm. daily for four to six months, 2 showed moderate roentgenographic improvement, 2 showed slight roentgenographic improvement, one showed no change, and none showed worsening. The hemogram remained stable in this group. Three patients continued to discharge tubercle bacilli, one had been noninfectious at the onset of therapy, and in one patient tubercle bacilli disappeared from the sputum during treatment.

In summary: A total of 23 patients with tuberculosis has been treated with S-ethyl-L-cysteine. The drug was well tolerated in doses of 4.0 gm. daily by mouth (1.0 gm. four times daily). No evidence of injury to bone marrow, liver, or kidneys was detected. There was no clinical intolerance to the drug except for the unpleasant odor to the breath. This odor was probably due to ethyl mercaptan, derived from cleavage of the compound by action of intestinal bacteria. Actually, it constituted a considerable obstacle to further use of this preparation.

Patients allocated to *Group I* were those with long-standing and essentially hopeless disease caused by tubercle bacilli resistant to other antimicrobial drugs. They received a maximum dose of 2.0 gm. daily of S-ethyl-L-cysteine. Benefit could scarcely be expected when failure had resulted from protracted treatment with isoniazid, streptomycin, and para-aminosalicylic acid; and little or no benefit was observed.

Patients listed in *Group II* also suffered from infection with tubercle bacilli resistant to other antituberculous drugs, but these received larger doses of S-ethyl-L-cysteine and there was suggestive evidence of temporary improvement due to the treatment.

Group III, consisting of 13 patients with previously untreated far advanced pulmonary

tuberculosis, provided more encouragement. Nevertheless, the rate of clinical, roentgenographic, and bacteriologic improvement was not equal to that expected from such established remedies as streptomycin and isoniazid.

Weimar Sanatorium
Weimar, Placer County, California

John A. Mathewson
Mildred Thoren

Stanford University School of Medicine
Stanford, California

H. Corwin Hinshaw

January 26, 1956

TRACES OF ZINC IN GLYCEROL[1]

Surface cultures of *Mycobacterium tuberculosis* (H37Rv) on Long synthetic culture medium[2] at pH 7.0, made with glass-distilled water, differ greatly according to the quality of the glycerol in the medium. Some lots of chemically pure glycerol are consistently associated with the production of quite scanty, scarcely wrinkled pellicles that soon become moist at 37° C., and other lots with profuse, much wrinkled, crisp, and dry-appearing pellicles that become wet only after longer incubation.

The surface cultures were produced by transplanting with a 0.4 cm. platinum loop thin pellicles of the bacilli growing on Long medium to the surface of 20 ml. of the same medium contained in especially cleaned 50 ml. Erlenmeyer Pyrex® glass flasks and incubating at 37° C. Definite results were obtained in four to five weeks.

Medium containing glycerol redistilled in Pyrex glass and kept in contact only with Pyrex glass has produced poor surface cultures. When 10^{-8}, 10^{-6}, and 10^{-5} per cent Zn^{++} as $ZnCl_2$ were added, the medium with the same glycerol produced profuse pellicles.

Poor growth-promoting chemically pure glycerol never produced a positive dithizone reaction for zinc[3] and always had its growth-promoting property much increased by the addition of as little as 10^{-5} per cent Zn^{++}.

TABLE I

DRY WEIGHTS OF H37Rv PELLICLES IN RELATION TO ZINC

Number of Flask*	Constant Dry Weight (Mg.)							
	Original Glycerol	Decationized Glycerol	Zn^{++} Per Cent Added with Decationized Glycerol					
			10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
1	253	23	32	59	160	228	229	228
2	247	21	38	59	218	214	231	221
3	252	24	31	58	216	225	229	226
4	Not done	22	25	40	224	235	234	225
Average	251	23	32	54	205	226	231	225

* Four flasks containing 20 ml. of Long medium (pH 7.0) for each group planted. Cultures removed from the incubator when they became slightly wet: thirty-three days for the decationized glycerol and the decationized glycerol with 10^{-6} and 10^{-7} per cent Zn^{++} ($ZnCl_2$), forty days for the others.

Chemically pure glycerol which promoted good growth was shown to contain traces of zinc by dithizone treatment. The good growth-promoting property was removed by passing the glycerol as a 20 per cent solution in de-ionized water through cleaned cation ion-exchange resin (Dowex® 50). The HCl eluate from the latter, when added to a deficient glycerol, made the latter more growth promoting.

In another experiment, a chemically pure glycerol, promoting profuse surface growth and containing about 3×10^{-4} per cent Zn^{++} according to a dithizone test,[3] was similarly

[1] This investigation was supported in part by Research Grant 3850 from the National Institutes of Health, Public Health Service.

[2] LONG, E. R., AND SEIBERT, F. B.: Am. Rev. Tuberc., 1926, *13*, 393.

[3] SANDELL, E. B.: Colorimetric Determinations of Traces of Metals, ed. 2, Interscience Publishers, Inc., New York, 1950, pp. 621–622.

decationized and used in the Long medium. Additions of 10^{-7} and 10^{-6} per cent Zn^{++} did not alter appreciably the appearances of the pellicles but did increase the dry weights of the pellicles. With 10^{-5} per cent Zn^{++} there was a pronounced increase of the thickness, wrinkling, crispness, and weights of the pellicles. However, the nonresin-treated glycerol promoted more growth than the decationized glycerol plus Zn^{++}, indicating that either the nontreated glycerol contained some other growth-promoting factor(s) or the resin contributed some inhibiting factor (table 1).

These results were obtained with L-asparagine. Traces of zinc did not produce these favorable effects with DL- and D-asparagine.

TUBERCULOSIS RESEARCH LABORATORY
GLOCKNER-PENROSE HOSPITAL
COLORADO SPRINGS, COLORADO

W. F. DREA

COLORADO FOUNDATION FOR RESEARCH IN TUBERCULOSIS
DENVER, COLORADO

February 24, 1956

THE INHIBITORY EFFECT OF PEPTONE ON THE SPORULATION OF THREE STRAINS OF COCCIDIOIDES IMMITIS[1]

In the course of other experiments on *Coccidioides immitis* it was frequently observed that some strains grew well but sporulated poorly when peptone was incorporated into the medium. Because peptone is so commonly used in media for various fungi, it seemed advisable to investigate this effect further.

Four strains were selected for testing. One of these was known to form spores prolifically and thus could serve as a comparative control. Two lots of media were prepared:

FIG. 1. *Coccidioides immitis* cultured on a glucose-yeast-extract agar containing peptone, showing inhibition of sporulation.

(*a*) 2 per cent glucose, one per cent yeast extract, and 2 per cent agar; and (*b*) the same, with one per cent Bacto-peptone added. One milliliter of saline suspensions of each of the four cultures was added to 20 ml. volumes of melted media and poured into Petri dishes with flat bottoms. Incubation was at 34°C.

The presence of peptone in the experimental media was associated with a varying degree of inhibition of spore formation in three of the four strains. This effect was not observed in the fourth strain. After approximately one week the mycelial mats of the strain showing marked inhibition were of the same size on both types of media. Two weeks later, however, the aerial hyphae of the culture of the glucose-yeast medium with peptone were

[1] This work was supported in part by a contract between the University of California, Department of Bacteriology, and the Office of Naval Research. The opinions contained in this report are not to be construed as reflecting the views of the Navy Department or the Naval Service at large (Article 1252, U. S. Navy Regulations, 1948).

FIG. 2. *Coccidioides immitis* cultured on a glucose-yeast-extract agar without peptone, showing excellent sporulation.

collapsed, leaving scattered areas of air trapped on the surface of the agar (figure 1). A microscopic preparation revealed that spores were present but sparse. On the glucose-yeast medium without peptone, cultures of this same strain showed grossly the cracked surface which is usually indicative of tremendous numbers of closely packed spores (figure 2). A microscopic preparation confirmed this and, in fact, revealed that all of the hyphae were almost completely converted to typical arthrospores.

The presence in peptones of substances inhibitory for pathogenic organisms has been reported by others.[2] The mechanism of the inhibition which we observed has not been determined nor has it been established that such an effect is characteristic of all peptones or even all lots of a single peptone. Nevertheless, in addition to stimulating interest concerning the nutritional requirements of this fungus, the common use of media containing peptone in laboratory practice warrants a brief report of these results. This is especially important since it has been shown that not all strains of *Coccidioides immitis* form spores in great numbers.[3]

NAVAL BIOLOGICAL LABORATORY
DEPARTMENT OF BACTERIOLOGY
UNIVERSITY OF CALIFORNIA
OAKLAND, CALIFORNIA

LORRAINE FRIEDMAN[4]
DEMOSTHENES PAPPAGIANIS

February 27, 1956

[2] McVICKAR, D. L.: J. Bact., 1951, *62*, 137.
[3] FRIEDMAN, L., *et al.*: J. Lab. & Clin. Med., 1953, *42*, 438.
[4] Present address: Tulane University School of Medicine, New Orleans, Louisiana.

INCIDENCE OF TUBERCULOUS INFECTION IN INFANCY[1,2]

There has been recent publicity to the effect that tuberculosis is no longer a disease menace. More accurately, it may be stated that, while mortality from this disease has markedly decreased, morbidity has been relatively unaffected. Certainly this is true in some sections of the United States.

At the Medical College of Virginia, Richmond, Virginia, there has been a small BCG study in progress for approximately six and one-half years.[3] Only infants less than six months of age, taken at random from the well-baby clinic of that institution, are admitted to this study. The cutaneous reaction to tuberculin is determined periodically thereafter both in the vaccinated and nonvaccinated infants and children.

The Mantoux test has been employed in testing this group. While Old Tuberculin solution (1.0 mg.) was employed in approximately one-half of the cases, the accuracy of this test was substantiated by the use of Old Tuberculin solution (0.1 mg.) in all except 19 instances. Follow-up visits could not be arranged for this number.

Of 791 control or nonvaccinated patients, 121 (15 per cent) have been infected with tuberculosis, as manifested by a positive reaction in the Mantoux test. One hundred and seven (13.5 per cent) were infected before they attained the age of three years and 86 (10.9 per cent) contracted tuberculous infection by the time they reached two years of age (table 1).

Of those infected with tuberculosis, none have died and only 2 have had complications of the disease. One of these (L.K.) developed tuberculous pneumonia and received isoniazid therapy for six months. Another (M.B.) with tuberculosis of one of the superficial cervical lymph nodes received isoniazid for four and one-half months and underwent uneventful excision of the affected node.

Since the patients included in the study were almost exclusively Negroes, it is not surprising that, among the 121 who showed a positive reaction to the Mantoux test, 119 were of that race.

The geographic distribution of the cases is interesting, but not significant (table 2). The small number of patients from the South Side of the city enrolled in the BCG program probably accounts for the fact that only a single case is listed from that area. Further, a tuberculin testing program recently instituted by the City Health Department in that section has been productive of a number of children infected with tuberculosis.

* * *

As Park[4] has observed, "There is more than one way of telling whether or not an adult has an open tuberculous lesion; one can find the bacilli in his sputum or one can find infection in his baby." The latter appears to be the case here. In table 3 it is shown that tuberculosis mortality in Richmond has decreased over a ten-year period as expected. On the other hand, morbidity has remained essentially unchanged (table 4). The high incidence of tuberculous disease among adults is reflected in the hitherto unsuspected

[1] Presented before the Medical Session, as part of *Section 3A*, at the annual meeting of the National Tuberculosis Association, New York, New York, May 22, 1956.

[2] This investigation was aided in part by a grant from the Richmond Tuberculosis Association.

[3] Figures from the BCG program include the period from November 1, 1949, to February 15, 1956.

[4] Holt, L. E., Jr., and McIntosh, R.: Holts' Diseases of Infancy and Childhood, ed. 11, D. Appleton-Century Company, Inc., New York, 1940, p. 1,276.

TABLE 1

AGE OF PATIENT WHEN POSITIVE TUBERCULIN REACTION NOTED

Age Group	Number of Patients
1 year or less	36
1 to 2 years	50
2 to 3 years	21
3 to 4 years	13
4 to 5 years	1
Total	121

TABLE 2

GEOGRAPHIC DISTRIBUTION OF CASES

Section of City	Number of Patients
East	38
West	33
Central	32
North	17
South	1
Total	121

TABLE 3

*DEATHS FROM TUBERCULOSIS, CITY OF RICHMOND, VIRGINIA**

1946–1955

Year	Number of Deaths	Deaths per 100,000 Population
1946	110	48.7
1947	115	50.9
1948	74	32.4
1949	107	46.6
1950	96	41.7
1951	78	33.4
1952	58	24.6
1953	40	16.8
1954	34	14.1
1955	20	8.4†

* Statistics as compiled by the Richmond City Division Public Health Statistics.

† Unofficial figures.

amount of tuberculous infection among infants in this study group. Obviously, widespread infection of infants and young children in this city will continue until the incidence of pulmonary tuberculosis among adults is curtailed.

The other interesting aspect of this study is the infrequency of complications in those infected during infancy. There were no deaths, and the 2 patients who experienced complications recovered promptly under appropriate therapy.

TABLE 4

REPORTED CASES OF TUBERCULOSIS, CITY OF RICHMOND, VIRGINIA*
1946–1955

Year	Number of Cases	Cases per 100,000 Population
1946	270	119.5
1947	206	91.2
1948	168	73.7
1949	168	73.2
1950	138	59.9
1951	187	80.2
1952	170	72.3
1953	205	86.2
1954	236	98.1
1955	235	98.4†

* Statistics as compiled by the Richmond City Division Public Health Statistics.
† Unofficial figures.

In summary: Tuberculosis morbidity continues to be a major problem. This is exemplified by the high incidence of tuberculous infection in infants enrolled as control patients in a BCG study in Richmond, Virginia. Statistics reveal that there is a commensurately high incidence of tuberculosis among adults in this city.

DEPARTMENT OF PEDIATRICS AND
CHILD CHEST CLINIC
MEDICAL COLLEGE OF VIRGINIA
RICHMOND, VIRGINIA

EDWIN L. KENDIG, JR.[5,6]

March 5, 1956

[5] Present address: 3603 Grove Avenue, Richmond, Virginia.
[6] The writer acknowledges the cooperation in this study of the Richmond City Health Department.

THE SEPARATION OF M. LEPRAE FROM TISSUES BY ENZYME DIGESTION

Several methods for the separation of *M. leprae* from tissues have been described, but each has certain inherent undesirable features. The method of Dharmendra (Leprosy in India, 1941, *13*, 77), which involves treating the tissue with chloroform, exposes the bacteria to reagents which may alter their viability, antigenicity, and specificity as well as their physical and chemical characteristics. During the procedure, large numbers of the organisms are lost in the discarded tissue sediment. Khanolka and Rajalashmi (Leprosy in India, 1952, *24*, 47) reported that the organisms could be isolated from tissues by ether extraction, but this method likewise subjects the organism to the deleterious effect of the reagents.

In an effort to obtain a tissue-free lepromin, preliminary studies indicated that trypsin could be effectively employed to separate tissue components from suspensions of *M. leprae* prepared from lepromata.

The following technique has been employed to obtain a tissue-free suspension of bacilli for use as lepromin. The lepromatous tissue is freed from gross fat and noninfected areas and then boiled for thirty minutes, following which it is finely ground with mortar and pestle in a minimal quantity of Sorenson's buffered saline with a pH of 7.6. Centrifugation at 400 r.p.m. for two minutes is carried out to eliminate large tissue particles. Subsequently the material is recentrifuged at 4,000 r.p.m. for ten minutes to remove tissue lipids. Sufficient Sorenson's solution containing 0.5 per cent trypsin (Difco Laboratories, 1:250) is added to the sediment to make a 1:20 dilution. The preparation is then incubated with frequent agitation at 37°C. for ninety minutes, following which any remaining tissue particles are removed by recentrifugation at 400 r.p.m. for ten minutes.

A suspension of tissue-free *M. leprae murium* obtained by digesting unboiled lepromatous tissue after grinding and exposure to trypsin digestion as above for two hours at 37°C. has produced typical murine leprosy. This procedure, however, is less effective than when the tissue is boiled.

The detection of *M. leprae* in suspected lesions for diagnosis is carried out in the same manner, except that the final boiled suspension is centrifuged for thirty minutes at 3,000 r.p.m. and the sediment is examined for acid-fast organisms. By this procedure, leprosy bacilli were concentrated from one to five bacilli in fifty microscopic fields to more than ten bacilli in a single field.

A tissue-free lepromin for the detection of leprosy should be more specific than preparations which contain extraneous tissue proteins, and an antigen containing only *M. leprae* can be more accurately standardized for diagnostic use. The method also makes available a tissue-free suspension of bacterial cells for more accurate biochemical and metabolic studies. Furthermore, the procedure provides a rapid practical means of concentrating *M. leprae* from biopsied tissue and facilitates its detection in the diagnosis of leprosy.

Department of Infectious Diseases
School of Medicine
University of California
Los Angeles, California

Joon Lew
Charles M. Carpenter

March 26, 1956

Correspondence

PERILS OF PROCRASTINATION IN PHTHISIOTHERAPY: URGENT INDICATIONS FOR ANTITUBERCULOUS MEDICATION

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

The efficacy, safety and availability of modern chemotherapy demand its administration in all cases of active tuberculosis as soon as the diagnosis is established. Even in the absence of bacteriologic proof, specific treatment for tuberculosis may often be required. In such cases adequate sputum, gastric or other specimens should be obtained for bacteriologic examination before antituberculous drugs are given which frequently and rapidly cause sputum conversion. Studies for cancer or other possible differential diagnoses should also be instituted without delay. In instances when unfavorable progression of the disease may be anticipated in the absence of specific treatment, however, withholding the drugs because of the absence of bacteriologic proof may be disastrous. Two fatal instances illustrate the seriousness of unduly deferring drug treatment pending verification of the diagnosis of active tuberculous lesions. Both of these patients suffered from forms of tuberculosis which are especially amenable to modern chemotherapy. The omission of specific medication for tuberculosis because of the absence of demonstrable tubercle bacilli in the sputum in these cases was therefore especially regrettable.

The first patient was a 67-year-old white laborer. He had pneumonia three years previously, treated with penicillin, and a chronic cough and weakness thereafter. A chest roentgenogram obtained because of increasing symptoms showed scattered nodular densities throughout both lungs, and the skin reaction to tuberculin was positive. However, several sputum specimens were negative for acid-fast bacilli on microscopic examination. No drug treatment was ordered while awaiting the results of sputum culture. The patient developed facial paralysis and speech disturbance ten days after admission and died two days later. Autopsy showed general miliary tuberculosis. Several weeks after the patient's death, an abundant growth of tubercle bacilli appeared in the sputum cultures obtained during life.

The second patient was a 69-year-old Negro laborer. A routine chest film in September, 1952, revealed pulmonary tuberculosis and a sputum specimen yielded tubercle bacilli. He was treated with isoniazid for eight months; all later sputum specimens were negative for tubercle bacilli and no chemotherapy for tuberculosis was given after July, 1953. On September 3, 1955, the patient was readmitted, acutely ill and dyspneic, complaining of substernal pain. A roentgenogram of the chest showed a greatly enlarged heart shadow. Electrocardiographic findings indicated auricular fibrillation and coronary arterial insufficiency. Under digitalization the fibrillation subsided, but the patient's condition deteriorated and he died October 18, 1955, six weeks after readmission. Autopsy revealed an extensive caseous tuberculous pericarditis.

A definite diagnosis of tuberculosis should not be made upon suspicion without seeking adequate confirmatory evidence. The mental distress from lingering uncertainty and the social maladjustment that it inflicts with the expense of verifying the diagnosis, the long and costly treatment, the disability and attendant economic loss during succeeding years, and the toxic side effects of the drug administered for long periods of time may not be lightly incurred. Efforts should always be made to establish the diagnosis of tuberculosis bacteriologically, but the absence of such proof should not deprive patients of benefits of needed specific treatment.

Antimicrobial drug therapy appears indicated in the following circumstances:

Active tuberculosis confirmed by recent bacteriologic demonstration of characteristic acid-fast bacilli or histopathologic findings typical of tuberculous lesions and not ascribable to fungi.

History of confirmed tuberculosis in the past, with recent clinical, roentgenographic or laboratory indications of active disease.

Acute disease threatening life unless immediately treated before diagnosis can be confirmed; as in miliary or meningeal tuberculosis suggested by clinical, roentgenographic or laboratory findings after at least one cerebrospinal fluid has been obtained for examination.

Extensive or increasing pneumonic disease with acute symptoms, history and roentgenograms consistent with active tuberculosis and not responding to sulfonamides, penicillin, or the tetracycline drugs after at least three sputum and/or gastric specimens are obtained for culture.

Patients with a positive tuberculin test and with roentgenographic abnormalities who give a history of contact, symptoms, or physical signs consistent with active tuberculosis. In such cases, the response to isoniazid and PAS may help to confirm the diagnosis after at least six adequate specimens have been studied for tubercle bacilli, including cultures, and extensive studies have failed to show other possibilities.

Eye, ear, gastrointestinal, genitourinary, skeletal, pericardial, pleural, peritoneal or extrapulmonary lesions after aspiration or other specimens are obtained since the isolation of tubercle bacilli is often difficult in such cases even when the disease is progressive.

Preliminary to chest surgery for resection of coin lesions or suspected tumors, or accompanying treatment with cortisone or corticotropin in patients with a positive tuberculin test when there may be suspicion of inactive or latent tuberculosis.

Primary tuberculosis which is recent or active as indicated by history, clinical findings or roentgenograms.

Positive tuberculin reactors who are recent converters with previous negative tests, or infants or children less than three years of age.

Negative reactors known to be heavily exposed under conditions in which removal or other forms of protection are not feasible, or when infection is known to have occurred, as following accidental or suicidal inoculation or inhalation of a culture of tubercle bacilli, et cetera.

Tuberculosis death rates have continued to decline, but are still unnecessarily high. Only too many of the fifteen thousand deaths from tuberculosis in the United States last year occurred because indicated chemotherapy had not been given. About a third of the fatal cases had not been reported as tuberculous before death, but even among the known nonhospitalized active cases, as reported by Dr. Blomquist in the *American Journal of Public Health* in February, 1956, a majority of the patients are not yet receiving the drugs. Even among patients in institutions, these effective agents are only too often omitted, or given too late, in inadequate regimens, or for insufficient time.

The deadly nature of tuberculosis and the failures that attend even the best modern treatment do not permit undue delay. Half of the persons who died with active tuberculosis in California last year had not received the life-saving treatments now available. Many of them missed this opportunity through failure of detection, but hesitation in diagnosis and timidity in therapy deprived only too many of their chance for survival. Deliberate withholding of treatment to elicit more convincing proof of the diagnosis succeeds only too often in worsening of the condition of the patient. A doctor who is "hell

on curing fits" should not therefore cause his patients to develop convulsions. It is better to cure without a diagnosis than to make it at the expense of the patient's safety.

Olive View Sanatorium
Olive View, California

Emil Bogen

February 27, 1956

MECHANISM OF THE EXACERBATION IN PULMONARY TUBERCULOSIS WITH SPECIAL REFERENCE TO ALLERGY

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

According to Choremis,[1] transitory exacerbation of fever and roentgenographic findings is often observed during streptomycin and isoniazid treatment of tuberculosis in children. It is stated in his paper that the exacerbation of the roentgenographic findings may be attributed to an intense exudative reaction which occurs under the influence of treatment with isoniazid-streptomycin, which might produce marked bacteriolysis and thus liberate tuberculin in a large amount, developing perifocal infiltrations and atelectasis. Oestreicher[2] also reported a similar phenomenon, attributing it to the diminished virulence of bacilli. We have also observed the same phenomenon in the pulmonary tuberculosis of adults, and have reported on it in detail. On the basis of investigations of experimentally produced pulmonary tuberculosis during the past ten years, we have formulated our own interpretation of this phenomenon, which attributes the cause to an allergic mechanism.

As we have reported,[3] when matured rabbits are inoculated by means of peritracheal instillation into one lung with a homogeneous solution containing 25 mg. of tubercle bacilli (freshly cultured from the sputum of a patient), the rabbits develop diffuse, unilateral, evenly concentrated shadows visible on the chest roentgenogram ten to fourteen days later. The animals autopsied in this stage showed tuberculous lobar pneumonia with necrosis. Three weeks after inoculation, roentgenographic examination showed involvement of the entire lung. In this latter stage, histologic examination revealed caseous pneumonia. After the fourth week, the animals developed cavities which progressively enlarged.

On the other hand, when the rabbits were previously sensitized by the bacilli and then inoculated as described above, the animals of the reinfected group showed a course different from that of the primary infection. The animals of the reinfected group developed an extensive, unilateral, evenly concentrated shadow visible on the chest roentgenogram two days after inoculation. Gross and microscopic examination revealed lobar tuberculous pneumonia. After the fifth week, the lesions gradually reduced in size and faded away, leaving histologically only the localized lobular productive lesions.

Comparing the pneumonic lesions in the reinfected animals with those of the primarily infected animals, the histologic pictures were almost the same but the lesions developed more promptly in the reinfected group. The characteristic histologic picture of allergy such as the vascular changes, i.e., the fibrinoid degeneration of the intima or media, was not recognized in these reinfected animals. One may demonstrate roentgenologically the

[1] Choremis, C. B., *et al.*: Am. Rev. Tubere., 1955, *72*, 527.
[2] Oestreicher, R.: Am. Rev. Tubere., 1955, *71*, 390.
[3] Kitazawa, Y.: Chiba M. J., 1953, *29*, 183.

prompt development of lesions in reinfected animals which may be interpreted as representing a Koch phenomenon.

In 1950, we published observations[4] on the roentgenologic response to chemotherapy in experimentally produced lesions. When nonsensitized animals were given PAS immediately after the first peritracheal inoculation of tubercle bacilli, no shadows developed. However, if the animals were given PAS after the development of extensive shadows, the latter were not reduced but enlarged further and eventually there was cavity formation. Similar results were obtained in later experiments with the administration of isoniazid.[5] On the other hand, in the reinfected group, when the animals were given isoniazid immediately after reinoculation, they developed extensive concentrated shadows visible on the roentgenogram two days later without any differences from the nontreated control group. This was reported in 1953 by one of our co-workers, H. Kamiyama.[5, 6] We also observed that, even if the animals were given streptomycin[7] immediately after reinoculation and PAS-isoniazid[8] several hours prior to reinoculation, similar results were obtained.

Concerning the marked difference between the response of both groups to chemotherapy, we have the following interpretation: In the primary infection group, the appearance of the shadows on the roentgenograms was inhibited due to the direct action of the drug on the tubercle bacillus. In reinfection, the allergic reaction due to the antigen-antibody response accounts for the prompt development of lesions after inoculation. It is not influenced by antituberculous agents because the inflammation is not specifically due to the bacteria, but is dependent upon the allergic response. If the early development of shadows were dependent upon the bacilli themselves, then their appearance could be inhibited by the antituberculous agents. These drugs cannot alter an allergic reaction and thus cannot inhibit the appearance of the early shadows.

When treatment of the reinfected group was continued, the extensive concentrated shadows which were demonstrated in the early stage were reduced more promptly and more completely than in the nontreated reinfected control group. Histologic examination of the treated group two months after reinoculation showed more complete reabsorption of the lesion, encapsulation of caseous foci, or fibrosis than in the corresponding nontreated control groups. We consider that the combination of acquired immunity and chemotherapy may account for such results in the reinfected animals. These experiments were confirmed by Mitsui[9] and Tanaka.[10]

Most of the research undertaken to evaluate the effect of the antituberculous drugs in humans has dealt with primary infection in animals. Since most cases of human tuberculosis are of the reinfection type, and since there exists such a marked difference in the response to antituberculous drugs between the primarily infected and the reinfected animals, further investigation on the effect of the antituberculous drugs in reinfection tuberculosis becomes necessary.

Our investigation[11] of clinical charts at Shoraiso Sanatorium revealed that 41 of 50 patients with exacerbation (82 per cent) manifested fever. Some of the chest roentgenograms, which were taken as early as three days following the onset of fever, revealed sha-

[4] Kitazawa, Y., *et al.*: Trans. Soc. path. jap., 1951, *40*, Editio Regionalis, 368.
[5] Kamiyama, H., *et al.*: Trans. Soc. path. jap., 1953, *42*, Editio Generalis, 574.
[6] Kamiyama, H.: Chiba M. J., 1953, *29*, 300.
[7] Shimada, T.: Trans. Soc. path. jap., 1953, *43*, Editio Generalis, 79.
[8] Ota, S., *et al.*: Trans. Soc. path. jap., 1954, *43*, Editio Generalis, 79.
[9] Mitsui, S., *et al.*: Kekkaku, 1954, *29*, 261.
[10] Tanaka, M.,: Kekkaku, 1954, *29*, 261.
[11] Kitazawa, Y., *et al.*: J. Jap. Soc. intern. Med., 1954, *43*, 739.

dows of infiltration. The time interval between intrabronchial spread and the development of symptoms in reinfection tuberculosis is usually unknown; that between infection and the development of symptoms in primary cases is also unknown. Thus, any conclusion regarding the role of allergy in reinfection tuberculosis based on a comparison of the above time intervals is not valid. In our experiments in reinfection, the animals showed early roentgenographic shadows even under chemotherapy. We found a similar phenomenon in patients during 1953. Seven patients in our sanatorium developed an exacerbation in spite of treatment with streptomycin-PAS. Twenty-seven patients who were treated with streptomycin-PAS after exacerbation (especially 9 patients who were treated with streptomycin-PAS immediately (within two weeks) after the exacerbation) were compared with the above-mentioned 7 patients. No significant differences in the progress of the roentgenographic findings resulted. The lesions of both groups reduced in size or disappeared under chemotherapy. The same results were obtained in the treatment of reinfected animals.

Occasionally, some writers have stated that exacerbation under chemotherapeutic controls is caused by drug-resistant bacilli, especially streptomycin-resistant strains. In our series, only in 2 of 6 cases did resistance to more than 10 γ of streptomycin occur. In the remaining 4 cases, the tubercle bacilli were susceptible to streptomycin. Therefore, one cannot ascribe exacerbations to the development of drug-resistant bacteria. Also, the roentgenographic shadows in the above 5 cases reduced in size or disappeared under streptomycin-PAS therapy.

The following questions should be answered: Were the exacerbations which occurred under the treatment with streptomycin-PAS actually recurrences of the pulmonary tuberculosis or not? Were they nontuberculous transitory infiltrations? These infiltrations persisted for several months before complete resorption. The clinical symptoms such as fever promptly disappeared and the general condition of the patients was markedly improved by the administration of streptomycin-PAS. In 5 of 6 cases, the number of tubercle bacilli in the sputum increased. Thus we conclude that the above-mentioned roentgenographic shadows were due to the exacerbation of pulmonary tuberculosis.

Since the number of tubercle bacilli found in the sputum decreases under chemotherapy, the possibility of exacerbation should also decrease. Even under chemotherapy, some patients develop pleurisy with effusion, tuberculous meningitis, or miliary tuberculosis. If these complications are interpreted as exacerbations, the mechanism of their development would be easily explained. That is, if their development is based on an allergic mechanism, then they naturally could occur even under treatment. Tuberculous meningitis or miliary tuberculosis differs from the exacerbation mainly in hematogenous dissemination as the reinfecting mechanism.

The exacerbation which occurs under chemotherapy is usually ameliorated by further continuation of the therapeutic regimen. This appears to be an inconsistency. However, it is borne out by experimental and clinical observations. This may give us the key to the solution of various problems in the pathogenesis of tuberculosis.

Department of Pathology
Chiba University School of Medicine,
Shoraiso Sanatorium of Health Insurance
Chiba, Japan

Yukio Kitazawa

August 29, 1955

DIAGNOSTIC STANDARDS AND CLASSIFICATION OF TUBERCULOSIS, 1950

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

There are some points in the NTA Classification of Pulmonary Tuberculosis, 1950 edition, which, I think, deserve discussion:

1. The Clinical Classification does not include planigraphy among the roentgenologic requirements for the diagnosis of the *inactive* status. This omission constitutes a definitely weak point, since planigraphy represents a *conditio sine qua non* for the correct evaluation of an *inactive* status of pulmonary tuberculosis.

2. The second group of the Clinical Classification, *arrested*, is not quite clear, and, in fact, is misleading. It is stated that "The symptomatic and roentgenologic requirements of this group are the same as for 'inactive'. When sputum specimens or gastric contents have been found negative only by microscopic examination of concentrates and not by culture or animal inoculation, such patients cannot be classified 'inactive'." The definition would be acceptable if it were limited to this statement. But it is widened so that "They [the patient's lesions] may be classified 'arrested' even though culture or animal inoculation may be positive and, among many concentrated specimens of sputum examined microscopically, an occasional positive may be found." The presence of tubercle bacilli in sputum specimens or gastric contents, as demonstrated by culture or animal inoculation, or even by microscopic examination of concentrated sputum, indicates that the disease is not completely *arrested*. It seems strange, I think, that pulmonary tuberculosis should be classified as *arrested* when bacilli are still being eliminated. But even from a linguistic viewpoint, the term *arrested* does not seem adequate. According to Webster's Dictionary (The World Publishing Company, 1953) *to arrest*, as used in the NTA Classification, means *to obstruct, to stop to check* or *hinder the motion of, as to arrest the current of a river*. Now, the current of a river can be arrested in the vicinity of its origin. In phthisiological language, pulmonary tuberculosis can be arrested in an early phase, when the extent of the lesions is minimal, and, in this case, arrest will mean, in the great majority of the patients, an *inactive* status, according to the NTA nomenclature. On the other hand, a current of a river can be interrupted just before it discharges into an ocean, a lake or another river. In phthisiological language, pulmonary tuberculosis can be arrested when the extent of the lesions is far advanced and, in this case, the patient is far from the *inactive* status. As a matter of fact, the arrest is, in most cases, only temporary and the patients become *good chronics*, inasmuch as the group of *arrested* does not exclude those who eliminate tubercle bacilli. It is clear, then, that the same term *arrested* can be applied to two widely different clinical and roentgenologic situations. The usefulness of this group is still further contradicted by the retrogressive status of the *active* group (*active: stationary, retrogressive or progressive*). Is there, really, a sharp distinction between *arrested* and *retrogressive?* I think there is not. An *arrested* pulmonary tuberculosis, in the NTA Classification, is merely one that has reached such a degree of retrogression that it is nearly *inactive*. Furthermore, an *arrested* disease, as defined by the NTA, does not represent a stabilized but rather a fluctuating situation: either the retrogression continues and the inactive status is reached, or an exacerbation occurs and the disease becomes progressive. In the light of these facts, would it be too great a disadvantage to simply discard this group?

3. Another criticism which should be made, and this is, certainly, not the least important, is that the Classification does not recognize the curability of tuberculosis. Not many lung specialists would agree with the restrictive concept of the expression *apparently cured* included in the 1940 edition of *Diagnostic Standards:* although the clinical, bac-

teriologic and roentgenologic examinations clearly indicated that a cure had been obtained, the patient would be classified as *apparently cured* for the rest of his life. And so, for instance, a person who, after a successful treatment, needed a health certificate to get a job or to be readmitted to his old one, would meet with great difficulties, because the employer would not be able to understand that a person *apparently* cured of tuberculosis could carry out normal work or adapt himself to a kind of work compatible with his new physical condition. The old skepticism of the laity toward the cure of tuberculosis was strengthened by the restriction imposed on the concept of the cure by the medical profession and the former patient found it difficult to return to normal life.

But the revision made in 1950 is still worse, because the word *cure* has been suppressed altogether, giving place to the *inactive* status. The crucial question is to ascertain whether we have or have not means sufficiently dependable and safe to assert the cure of pulmonary tuberculosis. The answer is affirmative in many instances.

I do not deem it necessary to mention the numerous and well-known bacteriologic and anatomic studies that plainly indicate the sterilization of tuberculous lesions. In this connection, it is well to remember that tuberculosis does not necessarily progress in a series of exacerbations and remissions. The clinical episode that brings a person to medical care can be the only one in his whole life and, following treatment, his condition can be precisely identical to that of an individual who has overcome a primary infection and reacts to tuberculin as the only sign of the infection. It is also well known that tuberculostatics have increased the possibility of cure. Consequently, there is, in many instances, no valid reason to prevent us from classifying a former patient as *cured*, simply *cured*, without any additional restrictive qualification which does not do justice to the result of the treatment and which needlessly complicates the life of the former patient.

Very sincerely yours,
José Feldman

Cadeira de Tisiologia
Faculdade de Medicina da
Universidade de Minas Gerais
Belo Horizonte, Minas Gerais
Brasil

March 9, 1956

Books

VACCINATION AGAINST TUBERCULOSIS. By L. Šula and co-workers. Collection of communications presented at the International Congress of the Pneumological and Phthisiological Society, Section of the Czechoslovak Medical Association of J. E. Purkyně, November 29–December 1, 1953, Státní Zdravotnické Nakladatelstvf, Prague, 1955.

The book contains summaries in Czech, Russian, and English of some thirty-four papers presented at an international congress in Prague in 1953. A majority of the papers outline the historical development and present status of the BCG vaccination programs in countries represented at the Congress: Bulgaria, China, Czechoslovakia, German Democratic Republic, Hungary, Poland, Rumanian People's Republic and U.S.S.R.; the remainder deal with various laboratory aspects of the production of fresh and freeze-dried vaccines, the relation of post-vaccination tuberculin allergy to immunity in tuberculosis, and recent laboratory and field experiences with the M-vaccine prepared by Šula from a murine strain derived from Wells' original vole bacillus.

Both fresh and freeze-dried BCG are commonly used, generally by intracutaneous injection of vaccine containing 0.5 mg. moist-weight organisms per ml. The age of the cultures for preparing vaccine is recommended variously by different laboratories as ten, fourteen, fifteen, and twenty-one days. Lyophilized vaccine is reported from East Germany to have no advantages over fresh vaccine and is not recommended, while dry vaccine is used in the U.S.S.R. and given subcutaneously rather than intracutaneously "because of its smaller local reaction." In Rumania a 20-prick instrument is used. Oral vaccination is apparently gaining in popularity for newborns, from 30 to 150 mg. for a single dose or 100 mg. for three repeated doses.

Conversion rates following intracutaneous vaccination are generally reported to be high, around 90 to 100 per cent, while with oral vaccination they range from around 45 to 50 per cent in Czechoslovakia to 95 per cent in China. Since 1947, Šula's BCG test, performed with intracutaneous BCG vaccine diluted 1:10 and killed by heat, has largely replaced the tuberculin test in Czechoslovakia: it is reported to be more sensitive than Mantoux I and II. Moreover, in persons revaccinated by the oral method, the percentage of positives to the BCG test is said to go up while the percentage of tuberculin positives goes down. The Trambusti test is now being tried in Rumania where the techniques of pre-vaccination testing are reported to need simplification.

Vaccination is now compulsory, particularly for the newborn, in many of the countries represented at the Congress, with periodic revaccination of nonreactors at frequent intervals. The beneficial effects of vaccination are highly acclaimed and local complications are reported as rare.

Resolutions passed by the Congress include the statement that vaccination is the most successful method in the fight against tuberculosis, and results in a rapid decline of the tuberculosis morbidity and mortality rates among the vaccinated as well as the nonvaccinated. The resolution also states, however, that serious deficiencies exist in the application of vaccination; that laboratory and technical equipment for research and production of vaccine are insufficient; that records on the efficacy of BCG vaccination are not complete and records of vaccinated persons and also of tuberculin tests are not in order; that watch must be made for complications of vaccination, especially if they do not clear up within three months; that new ideas on tuberculosis immunity, arising from the teachings

of Pavlov, will help in future activity; and, finally, that "particular attention should be paid to the variability of the micro-organisms, endeavoring in this way to find a more efficacious vaccine against tuberculosis."

While the material given in the book is necessarily brief, the publication nevertheless brings together information and opinions from various countries from which little has been available in recent years. Problems with BCG in the Eastern countries have apparently not been very different from those in the West. Low degrees of post-vaccination allergy have inspired the search for a test more sensitive than even large doses of tuberculin in order to demonstrate that vaccination has been satisfactory (despite the assertion that the degree of allergy parallels the degree of immunity). Revaccination is considered necessary, and in some countries is compulsory, at three- to four-year intervals from infancy to young adulthood. The value of BCG is widely acclaimed along with demands for a more effective vaccine.

Carroll E. Palmer
Washington, D. C.

Studies in Tuberculosis. By R. G. Ferguson. Pp. 124, University of Toronto Press, Toronto, Canada, 1955, Price $3.50.

The author has written a compact and fascinating book on some epidemiologic aspects of tuberculosis which stem from a series of studies he and his colleagues of the staff of the Saskatchewan Anti-Tuberculosis League conducted for the purpose of clarifying some debated points with respect to tuberculosis problems. The text, which is written in a direct and simple style, will be especially interesting to students of tuberculosis and epidemiology. In addition to the presentation of his own studies, the author has also summarized some of the relevant literature on the particular subject under discussion.

This book is divided into four parts: epidemiologic factors in tuberculosis, infection and resistance, the course of epidemics, and the foundations of a preventive program. A useful addition of appendices and a bibliography is also included.

The author is careful to separate his own opinions and deductions from the facts in any given clinical study which he records. The book is not, of course, designed to be a comprehensive review of the various topics which are discussed, so that understandably some chapters contain only brief summaries; this is especially true of the chapter on rehabilitation wherein only the general principles and approaches are mentioned.

One of the very real attributes of this volume is that it does represent a distillation of the studied beliefs and conclusions of a disciplined and competent observer who has a professional lifetime experience in the study of tuberculosis problems.

Kurt W. Deuschle
New York, New York

Annual Review of Medicine. Volume 6. Pp. 450, Annual Reviews, Inc., Stanford, California, 1955, Price $7.00.

Once again this useful volume successfully meets its objective and makes a major contribution to a solution of the problem of keeping abreast of advances in medicine as reported in the literature.

The internist will find especially valuable some of the reviews of advances outside his usual immediate field of interest; for example, the survey of newer diagnostic methods in roentgenology, the review of recent advances in ophthalmology, and the paper on recent developments in psychiatry.

Of additional and particular interest are Scott's review on viruses, Professor Alexander Haddow's review on cancer (including a moderate, well-reasoned statement on the tobacco-lung cancer controversy), and the section on liver disease by Bearn and Kunkel.

The list of annotated reviews in medicine continues to be a useful feature of this series.

This volume is recommended for students and physicians as one which contains something of use for all.

JEREMIAH A. BARONDESS
New York, New York

BOOKS RECEIVED

ABILITIES, BASIC CONCEPTS, AND CONTENT IN TUBERCULOSIS FOR PUBLIC HEALTH NURSES. Report of Tuberculosis Conference for Public Health Nurse Faculty Members. Pp. 54, National League for Nursing, Inc., New York, 1956, Price, $1.00.

A VENTURE FORWARD. A History of the American College of Hospital Administrators. Compiled by Ira A. Kipnis. Pp. 145, American College of Hospital Administrators, Chicago, 1955, Price, $5.00.

PUBLICACIONES DEL CENTRO DE INVESTIGACIONES TISIOLOGICAS. Volume XVIII. Edited by Roque A. Izzo. Pp. 266, Policlinico Tornu, Buenos Aires, 1954–1955.

TUBERCULOSE UTÉRO-ANNEXIELLE. Aspects Actuels—Diagnostic Traitement. By Jules Bret and Robert Legros. Pp. 147, Masson & Cie, Paris, 1956, Price, 1,600 fr.

CONTROL DE LA TUBERCULOSIS EN PUERTO RICO. By Fernando Padró. Pp. 71, Departmento de Salud, San Juan, 1956.

PULMONARY CARCINOMA. Pathogenesis, Diagnosis, and Treatment. Edited by Edgar Mayer and Herbert C. Maier. Pp. 540, New York University Press, New York (distributed by J. B. Lippincott Company, Philadelphia), 1956, Price, $15.00.

THERAPY OF FUNGUS DISEASES. An International Symposium. Edited by Thomas H. Sternberg and Victor D. Newcomer. Pp. 337, Little, Brown and Company, Boston, 1955, $7.50.

LES FONDEMENT ANATOMO-RADIOLOGIQUES DE L'INVESTIGATION PULMONAIRE. By F. Kovats and Z. Zsebok. Pp. 336, Masson & Cie, Paris, 1955, Price, 8,000 fr.

American Trudeau Society

Medical Section of the National Tuberculosis Association

Obituaries

WILLIAM HERBERT ORDWAY

1889–1955

Dr. William Herbert Ordway died in the Springfield (Massachusetts) Hospital on Friday, April 1, 1955. Myocardial disease had hampered his natural enthusiastic mode of living for many years.

Dr. Ordway was born in Chicopee Falls, Massachusetts, on March 24, 1889, received his Doctorate in Medicine from Yale University in 1912, served an internship in City Hospital, New York City, and later was an assistant at the Potter Metabolic Clinic. In 1917 he enlisted in the U. S. Army, was stationed first at Camp Mills, later at General Hospital No. 9 at Lakewood, New Jersey, until he went to France.

In 1919 Dr. Ordway joined the medical staff at the Metropolitan Life Insurance Company sanatorium at Mount McGregor where he held the posts successively of Assistant Physician and Associate Physician, until 1926 when he was appointed Assistant Medical Director and Physician-in-Charge of the sanatorium.

He married Miss Hazel Cook of Chicopee Falls in 1916 and was an attentive and devoted husband until her death in January, 1947. In July, 1951, Dr. Ordway married Miss Wesleyna Smith who survives him.

Dr. Ordway was an enthusiastic conscientious physician who gave meticulous attention to all aspects of the care of the sick. He sought always to improve himself, the staff for which he held himself responsible, and the sanatorium, which he loved. He was President of the Saratoga County (New York) Medical Society from 1929–1931; a consultant at the Saratoga General Hospital, and a trustee and President of the Board of Directors of the Potts Memorial Hospital. In 1931 Dr. Ordway was elected Secretary-Treasurer of the American Sanatorium Association, in 1934 he became Vice-President, and President in 1935. Another contribution to public welfare was his service to the Saratoga County Tuberculosis and Health Committee, of which he was Director and President in 1936–1937.

In 1936 he was elected a Fellow of the American College of Physicians and was Chief Medical Officer of the Saratoga Civil Defense Committee in World War II. In 1948 he was elected to the presidency of the Yale University Medical Association and of the Yale Student Loan Fund. This latter activity was a natural outlet for his interest in young people. While a member of the Rotary Club of Glens Falls, New York, Dr. Ordway showed his generosity and kindness by helping to improve the lot of underprivileged children.

In spite of his many and diverse activities, Dr. Ordway was an avid student and observer who contributed many original articles to the medical literature. Dr. Ordway worked untiringly on, and contributed much to, the development and acceptance of the diagnostic codes in the Section on Diseases of the Bronchi, Lungs, and Pleura in the *Standard Nomenclature of Diseases and Operations*. His keen interest in raising the standards of medical care resulted in the formation of the Trudeau Society of Mount McGregor. Outstanding physicians and surgeons from the East were guest speakers before this group which encouraged attendance by all of the general practitioners within motoring distance of Mount McGregor.

To his friends Dr. Ordway was a gracious and charming host. He enjoyed a few hobbies,

such as woodworking and model railroading, and was at his good, natural and chuckling best when he shed the responsibilities of his official life to play with his associates. His life was one of high personal integrity and of conscientious service to the sick. However, he is remembered best by his friends as a true and loyal companion.

H.H.F.

C. H. LERRIGO

1872–1955

Dr. C. H. Lerrigo, executive secretary emeritus of the Kansas Tuberculosis and Health Association, died recently. Although his visits to the Association became less frequent during his later years, they were always an inspiration to those workers in the field of public health.

Dr. Lerrigo was born in Birmingham, England, in 1872. He came to the United States with his family and settled in Morris County, Kansas, in 1885. He was graduated from The Homeopathic Medical College, St. Louis, in 1900 and received his medical license in 1901, whereupon he established himself as a family doctor in Topeka.

He soon became interested in public health work and, because of his interest, Governor Hoch, in 1905, appointed him a member of the Kansas State Board of Health on which he served until 1923.

During World War I, Dr. Lerrigo served as commanding officer of Army Ambulance Company 347, which he was instrumental in organizing from the student body at Washburn College. This company saw oversens service in France

As early as 1906, when tuberculosis was claiming more than one thousand lives each year in Kansas, Dr. Lerrigo recognized the importance of the tuberculosis problem and was one of the persons most responsible for the organization of the Kansas Society for the Study and Prevention of Tuberculosis, serving as a volunteer worker and officer from 1908 until 1919.

This interest in tuberculosis caused Dr. Lerrigo to resign his position as State Registrar in 1922 and to accept the position as executive secretary of the Kansas Tuberculosis and Health Association, a position which he held until his retirement in 1948. During this same year, Dr. Lerrigo was honored for having made an outstanding contribution to the public health field in Kansas. He received this honor at the annual meeting of the Kansas Public Health Association.

Although officially retired, Dr. Lerrigo continued to write and his last book, *The Better Half of Your Life*, was published in 1951 when he was seventy-nine.

In 1937, Dr. Lerrigo wrote to the volunteer tuberculosis workers in Kansas that, although they were being called visionaries, they should not take this term as one of reproach. "In our sense of the word it is good to be a visionary. It may never bring you lucre but it lifts you into the ranks of those who are doing something—going somewhere."

In these words, Dr. Lerrigo described his own courageous spirit which he expressed in his dedication to the cause of better public health and the eradication of tuberculosis in Kansas.

WALTER J. MARCLEY

1867–1955

Dr. Walter J. Marcley, one of the original founders of the National Tuberculosis Association, died on November 8, 1955.

Dr. Marcley was born on September 21, 1867, in Franconia, Minnesota. He was graduated from the University of North Dakota and, in 1895, from the Boston Medical School.

In the spring of 1897, Dr. Marcley was appointed first superintendent of the Massachusetts Sanatorium at Rutland, the first state sanatorium in the United States. He became the first superintendent of Minnesota State Sanatorium from 1907 to 1911, when he resigned to establish private practice in Minneapolis. For several years he served as attending physician at Hopewell Hospital, the tuberculosis section of the Minneapolis City Hospital.

He served as medical director of Glen Lake Sanatorium in 1916 and 1917. He had selected the site for this sanatorium and had worked with the local architects in planning the original buildings.

In July, 1917, he was ordered to duty as a contract surgeon at Fort Snelling; he was the first tuberculosis specialist in Minnesota to be assigned to detect tuberculosis in the Army. Upon his return from this duty, he organized the Veterans tuberculosis program and later became the first chief of the tuberculosis service in the Veterans' Hospital. Following his retirement from the Veterans Administration, he served as chief of the outpatient department of Nopeming Sanatorium and subsequently as tuberculosis consultant to the Minnesota State Department of Health.

In 1918 and 1919, he served with the Red Cross Tuberculosis Commission in Switzerland and France.

Dr. Marcley was married in July, 1905, to Jessie MacMillan, who survives him.

ALBERT J. CHESLEY

1877–1955

Dr. Albert J. Chesley, the Dean of Health Officers in North America, died on Monday, October 17, 1955, in Rochester, Minnesota, of myocardial infarction.

Dr. Chesley was born in Minneapolis, Minnesota, on September 12, 1877, where he spent most of his life.

After having served as private and corporal of the Thirteenth Minnesota Volunteer Infantry during the Spanish American War, he joined the Minnesota State Department of Health as laboratory assistant in 1901. He completed his pre-medical and medical education at the University of Minnesota Medical School, and received the degree of Doctor of Medicine in 1907. After graduation he was appointed assistant bacteriologist, later assistant epidemiologist and, in 1914, director of preventable diseases.

During World War I, Dr. Chesley served as public health expert for the American Red Cross in France. After the Armistice, he was assigned to Poland as Chief of Staff of the Red Cross Commission, and was later Commissioner to Poland. For his distinguished services he received the Medaille de la Reconnaissance Francaise 3eme classe and three high decorations from the government of Poland.

In 1921, he was appointed Secretary and Executive Officer of the Minnesota Department of Health, a position he held until his death. Among his noteworthy achievements in this position were advances made in the control of diphtheria, tuberculosis, and poliomyelitis. The close supervisory relations of the Department of Health in regard to the health and medical care of the American Indian population permitted a comprehensive study of the social, educational, and economic problems involved, and Dr. Chesley realized a life's dream when Congress transferred the health and medical care of the Indians to the Public Health Service in July, 1955.

Dr. Chesley was a Professor of Public Health, Department of Preventive Medicine, in the University of Minnesota, and was the recipient of the Tuberculosis Award of the Minnesota Public Health Association. Further high honors attest to his leadership in public health affairs: He was President of the American Public Health Association; President of

the Association of State and Territorial Health Officers; Honorary Fellow of the Royal Sanitary Institute of Great Britain; Honorary Life Membership Canadian Public Health Association; Honorary Life Member American Social Hygiene Association; and member of American Epidemiological Society, American Association of Pathologists and Bacteriologists, Board of Scientific Directors of the Rockefeller Foundation, and International Health Division.

Dr. Chesley was the recipient of the Distinguished Service Medal of the Minnesota State Medical Association; Achievement Award, University of Minnesota; and Arthur T. McCormack Award, Association of State and Territorial Health Officers. He was Diplomate, American Board of Preventive Medicine and Public Health, and a Fellow of the American College of Preventive Medicine.

Dr. Chesley lived "public health" for more than half a century—a symbol and memorial of sacrifice and devotion to human service.

NOTICES

PUBLIC HEALTH SERVICE RESEARCH GRANT APPLICATIONS

The Public Health Service has announced a new procedure to expedite the processing of research grant applications for those requests which do not exceed $2,000 plus indirect costs and which do not ask support for more than one year. Such applications will be accepted and processed on receipt and are not therefore subject to the usual deadlines for submission prior to review.

Council recommendations can be expected on these applications within one to four months from the time of submission. These procedures do not apply for requests for supplements to existing grants.

Address all applications as well as requests for forms or additional information to the Division of Research Grants, National Institutes of Health, Bethesda 14, Maryland.

FOURTH INTERNATIONAL CONGRESS ON DISEASES OF THE CHEST OF THE AMERICAN COLLEGE OF CHEST PHYSICIANS

The Fourth International Congress on Diseases of the Chest of the American College of Chest Physicians will be held in Cologne, Germany, from August 19 to August 23, 1956, under the patronage of the Federal Chancellor, Dr. Konrad Adenauer.

The main subjects which will be discussed at the Congress deal with the problems of coronary diseases (diagnosis, pathophysiology, and surgery), industrial diseases of the chest, tuberculosis, lung and heart function and tumors of the mediastinum. Several outstanding foreign and German scientists and clinicians will present papers on these subjects. This year's Congress will be held with special reference to surgery of coronary diseases, but presentations on any other subject in the field of diseases of the chest will also be accepted. The presentations will be followed by free discussion. The official languages for the Congress are: English, French, Spanish and German.

For more detailed information and inscription, please write to the secretariat of the Congress: Fourth International Congress of the American College of Chest Physicians, Köln-Deutz, Germany, Messeplatz.

SCHEDULE OF LABORATORY REFRESHER TRAINING COURSES

July, 1956–June, 1957

The following schedule of laboratory refresher training courses will be offered by the Laboratory Branch of the Communicable Disease Center, Chamblee, Georgia.

Information and application forms should be requested from Laboratory Training Services, Communicable Disease Center, U. S. Public Health Service, P. O. Box 85, Chamblee, Georgia.

Dates	Course Number	Cousres	Duration	Location*
			weeks	
Sept. 10–21	8.40	Laboratory Methods in the Diagnosis of Bacterial Diseases Part 1. General Bacteriology	2	2
Sept. 10–Oct. 5	8.00	Laboratory Methods in the Diagnosis of Parasitic Diseases Part 1. Intestinal Parasites	4	1
Sept. 24–Oct. 5	8.41	Laboratory Methods in the Diagnosis of Bacterial Diseases Part 2. General Bacteriology	2	2
Oct 8–19	8.50	Laboratory Methods in the Diagnosis of Bacterial Diseases, Enteric Bacteriology	2	2
Oct 8–26	8.01	Laboratory Methods in the Diagnosis of Parasitic Diseases Part 2. Blood Parasites	3	1
Oct. 15–26	8.20	Laboratory Methods in the Diagnosis of Viral and Rickettsial Diseases	2	3
Oct. 29–Nov. 2	8.26	Laboratory Methods in the Diagnosis of Rabies	1	3
Jan 7–18	8.15	Laboratory Methods in Medical Mycology Part 1. Cutaneous Pathogenic Fungi	2	1
Jan. 21–Feb. 1	8.16	Laboratory Methods in Medical Mycology Part 2. Subcutaneous & Systemic Fungi†	2	1
Jan. 21–Feb. 1	8.55	Laboratory Methods in the Diagnosis of Tuberculosis	2	2
Feb. 4–15	8.17	Laboratory Methods in the Study of Pulmonary Mycoses	2	1
Feb. 25–Mar. 1	9.40	Laboratory Diagnostic Methods in Veterinary Mycology	1	1
Mar. 11–22	8.20	Laboratory Methods in the Diagnosis of Viral and Rickettsial Diseases	2	3
Mar. 11–22	9.41	Serologic Methods in the Diagnosis of Parasitic and Mycotic Infections	2	1
Mar. 25–29	8.26	Laboratory Methods in the Diagnosis of Rabies	1	3
++	8.05	Laboratory Methods in the Diagnosis of Malaria	2	1
++	8.21	Virus Isolation and Identification Techniques	2–4	3
++	8.42	Typing of *Corynebacterium diphtheriae*	1	2
++	8.51	Special Problems in Enteric Bacteriology	2	2
++	8.52	Phage Typing of *Salmonella typhosa*	1	2
++	8.53	Laboratory Methods in the Diagnosis of Leptospirosis	1–4	2
++	8.54	Serologic Differentiation of Streptococci	2	2

* Location of classrooms:
1 Bldg. 27, Chamblee, Ga.
2 Bldg. 30, Chamblee, Ga.
3 Virus Laboratory, Montgomery, Ala.

† Completion of Course 8.15 or equivalent education or experience is a prerequisite.

++ Courses given by special arrangement only.

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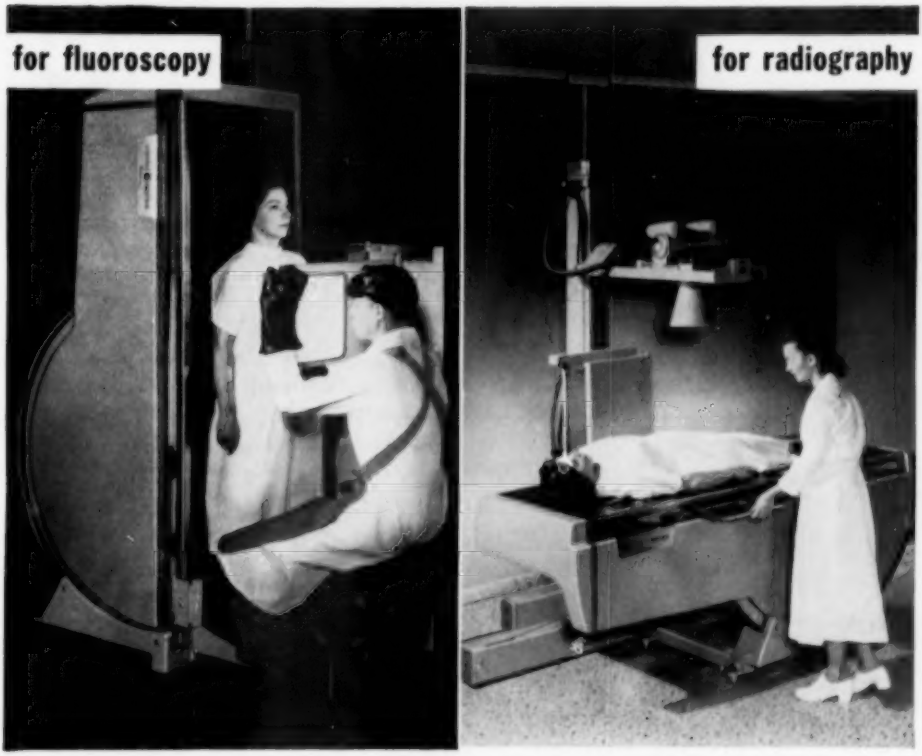

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American Trudeau Society

Medical Section—National Tuberculosis Association

1790 Broadway, New York 19, N. Y.

An organization of physicians interested in pulmonary and other thoracic diseases, with special emphasis on tuberculosis.

APPLICATION FOR ACTIVE MEMBERSHIP

Name _____ **M.D.**
(Print name in full—first, middle, last)

Mailing Address _____
(Street) (city or town) (state)

Place of Birth _____ **Date of Birth** _____
(city or town) (state or country)

Education

University _____ Year graduated _____

Medical School _____ Year graduated _____

Postgraduate Training _____

_____

Professional Societies of which a Member (local, state, and national) _____

_____

_____

_____

Type of Practice _____
(title of position)

_____
(name of organization) (part or full-time)

_____
(character of work—if private practice so state)

Endorsements (Two signatures of American Trudeau Society members are required.)
This applicant is personally known to me and I recommend his election to membership in the American Trudeau Society.
(print or type)

(a) Name _____

Address _____ Signature _____

(b) Name _____

Address _____ Signature _____

Membership

Active members shall have a medical degree. They may be specialists in thoracic diseases, physicians connected with tuberculosis hospitals or clinics, physicians in the public health field concerned with tuberculosis control, thoracic surgeons, research workers in tuberculosis, or they may be physicians interested in our objectives.

Candidates for active membership shall be recommended by the membership committee and elected by the Council, or the Executive Committee acting for the Council.

Dues

The dues of the members shall cover membership for the calendar year ($15.00) with the exception that those of new members may for the first six months cover from July to December, inclusive ($7.50).

Each active member will receive The American Review of Tuberculosis and Pulmonary Diseases for twelve consecutive issues. Members will also receive a subscription to the Bulletin of the NTA and to the Society's News Letter.

I will conform to the requirements of membership if elected.

Date: _____ Signature: _____ M.D.

_____
(Please print name here as you wish it to appear on your membership certificate)

Date Elected _____ Date Notified _____

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